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Citation for published version:
Crous, KY, O'sullivan, OS, Zaragoza-Castells, J, Bloomfield, KJ, Negrini, CA, Meir, P, Turnbull, MH, Griffin, KL & Atkin, OK 2017, 'Nitrogen and phosphorus availability interact to modulate leaf trait scaling relationships across six plant functional types in a controlled-environment study', *New Phytologist*. https://doi.org/10.1111/nph.14591

Digital Object Identifier (DOI):
10.1111/nph.14591

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
New Phytologist

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Download date: 07. May. 2023

Nitrogen and phosphorus availability interact to modulate leaf trait scaling relationships across six plant functional types in a controlled-environment study

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Number of Figures: ~~9~~5 (plus 5 in Supporting Information)
Number of Tables: 5 (plus 5~~4~~ in Supporting Information)
Number of References: 91~~7~~
Number of Pages (text plus references): 23

Total Word count: ~~6558~~ 6735 (excluding Abstract, References, Figures and Tables)
Abstract: 200 words
Introduction: ~~1178~~ 1513 words
Materials and Methods: ~~1048~~ 1002 words
Results: ~~2337~~2376 words
Discussion: ~~1938~~ 1788 words
Acknowledgements: 56~~7~~ words

Summary

- Nitrogen (N) and phosphorus (P) have key roles in ~~plant~~ leaf metabolism resulting in a strong coupling of ~~leaf~~ chemical composition traits to metabolic rates in field-based studies. However, in such studies, it is difficult to disentangle the effects of nutrient supply *per se* on trait-trait ~~scaling~~ relationships.
- Our study assessed how high and low N (5 mM and 0.4 mM respectively) and P (1 mM and 2 μM P respectively) supply on 37 species from six plant functional types ~~(PFT)~~ affected photosynthesis (*A*) and respiration (*R*) ([~~i~~in darkness ~~(R_{Dark})~~ and light ~~(R_{Light})~~]) in a controlled-environment.
- Low P supply ~~only reduced~~increased scaling-exponents (slopes) of area-based ~~the~~log-log ~~-~~*A*-N or *R*-N relationships when N supply was not limiting ~~(high N)~~, whereas there was no P-effect ~~in~~under low N supply ~~-grown plants~~. By contrast, scaling-exponents ~~slopes~~ of *A*-P and *R*-P relationships were altered by ~~both~~ P and N supply~~, with lower N resulting in lower metabolic rates for a given P~~. ~~While~~ ~~N~~nNeither ~~the~~ *R*:*A* ~~ratio~~ nor light inhibition of leaf *R* ~~the~~ R_{Light}:R_{Dark} ~~ratio~~ were~~as~~ affected by nutrient supply. Light inhibition ~~of respiration~~ was 26% across nutrient treatments; ~~but he~~herbaceous species ~~had~~exhibited ~~, though some PFT differences occurred.~~a lower degree ~~of~~ light inhibition than woody species. ~~Slopes of *A*-N and *R*-N relationships were lower in plants grown on low P and high N, whereas there was no P-effect in low N-grown plants. As a function of leaf P, slopes of bivariate relationships were altered by both P and N supply, with lower N resulting in lower metabolic rates for a given P. Neither the *R*:*A* ratio nor the R_{Light}:R_{Dark} ratio was affected by nutrient supply, though some PFT differences occurred.~~
- Because ~~variations in~~ N and P supply modulates leaf trait-trait relationships, ~~it is vital to characterise the mechanistic impact of nutrient supply *per se* on these relationships given their application in~~ the next generation of terrestrial biosphere models may need to consider how limitations in N and P availability affect trait-trait relationships when predicting carbon exchange ~~using leaf chemical composition need to incorporate nutrient variations across biomes~~.

Keywords: day respiration, glasshouse, leaf economic spectrum, nitrogen, nutrient supply, ~~phosphorus,~~ photosynthesis, plant functional groups.

Introduction

Nitrogen and phosphorus are both essential plant nutrients involved in many important plant metabolic functions, including rates of light-saturated photosynthesis (*A*) and leaf respiration (*R*). Strong positive relationships are found between both *A, R* and foliar N (Field & Mooney, 1986; Evans, 1989; Ryan, 1995; Reich *et al.*, 2008), reflecting the presence of N in key proteins. As a result, N deficiencies can ~~have negative effects on~~ reduce capacity of photosynthetic and respiratory enzymes, leading to lower leaf CO_2 exchange (Evans & Terashima, 1988; Noguchi & Terashima, 2006). Phosphorus (P) is a major component of nucleic acids, ATP, sugar phosphates and phospholipids, all of which are intrinsically related to photosynthesis and respiration (Rao & Terry, 1995). Consequently, P-deficient plants often exhibit: reduced stomatal conductance (Thomas *et al.*, 2006); reduced ribulose bisphosphate (RuBP) regeneration, carboxylation activity and *A* (Bloomfield *et al.*, 2014; Ellsworth *et al.*, 2015); and, lower allocation of N to RuBP carboxylase/oxygenase (Rubisco) (Warren & Adams, 2002). Increased N allocation to non-photosynthetic components and/or inactivation of Rubisco are additional factors that might contribute to lower *A* under P-deficiency (Stitt & Schulze, 1994). Phosphorus also plays a crucial role in regulating glycolysis and mitochondrial electron transport (Plaxton & Podesta, 2006), with low P supply having the potential to result in reduced *R* (Gonzalez-Meler *et al.*, 2001). Limitations in P supply can lead to accumulation of starch (Hammond & White, 2008), ~~and~~ increases in dry mass per unit leaf area (M_a) (Fyllas *et al.*, 2009; Turnbull *et al.*, 2016). Importantly, the effect of P-deficiency on ~~such~~ plant traits is likely to differ depending on whether P is more or less limiting than N~~, reflecting Liebig's law of the minimum~~ (e.g. Aerts *et al.*, 1992; Meir *et al.*, 2001)~~(Ågren *et al.*, 2012).~~

The coupling of metabolism to leaf chemical composition is important for predictions of plant carbon exchange at a global scale ~~(Sitch *et al.*, 2008)~~ (Kattge *et al.*, 2009), with ~~'scaling relationships' having been developed linking~~ variation in metabolic rates being linked to variation in~~to~~ M_a and mass-based leaf N (N_m) (Wright *et al.*, 2004; Kattge *et al.*, 2011). Plants growing on nutrient-poor soils often have longer lived leaves, higher M_a values, lower N_m, and lower mass-based *A* (A_m) and leaf *R* in darkness ($R_{Dark,m}$) than plants growing on nutrient-rich soils ~~(Wright & Westoby, 2003)~~ (Wright *et al.*, 2003; Wright & Westoby, 2003). When trait-trait relationships are compared for sites differing in nutrient availability, distinct patterns are often observed. For example, the slope of ~~bivariate~~ linear ~~relationships~~ regressions linking *A* to leaf N_m are often lower

in plants growing on low P-soils (Reich & Walters, 1994; Denton *et al.*, 2007; Kattge *et al.*, 2009). Comparing tropical leaf traits at sites differing in P availability, Kattge *et al.* (2009) reported lower rates of area-based maximum carboxylation rates ($V_{cmax,a}$) at given area-based N (N_a) in plants growing on low P soils. Similarly, the slope (or scaling-exponent) of ~~the~~ log-log *A*-N relationships is lower in tropical trees growing on low P soils than temperate trees growing on high P soils (Reich *et al.*, 2009). ~~Moreover,~~ Bahar *et al.* (2016) reported lower N-based rates of *A* and V_{cmax} in tropical rainforest species growing on low P soils in Peru (compared to high P soils). Collectively, these field-based studies suggest that coupling of photosynthetic metabolism with N may depend on P-availability ~~and hence a single 'universal' scaling relationships may not be realistic~~.

Relationships between leaf N and dark respiration are also affected by P-supply. Meir *et al.* (2001) found that area-based leaf R_{Dark} ($R_{Dark,a}$) at a given N_a being higher at P-limited sites. By contrast, Rowland *et al.* (2016) reported higher ~~area-based R_{Dark} ($R_{Dark,a}$)~~ at a given N_a in tropical trees growing on low nutrient soils; this study also found that $R_{Dark,a}$ was less affected by nutrient limitations than *A*, resulting in higher *R:A* ratios in plants growing on nutrient-deficient soils. Further, Reich *et al.* (2006) found that the log-log relationship between whole-plant $R_{Dark,m}$ and N_m was unaffected by nutrient supply, a finding also observed at the leaf-level (Atkin *et al.*, 2013). In comparisons of log-log plots, Wright *et al.* (2004) showed ~~intercept~~ elevation (proportionality-coefficient) differences ~~for~~ among sites differing in nutrient availability but with constant ~~-~~$R_{dark,m}$-N_m ~~slopes~~ scaling-exponents across sites. Notwithstanding the contrasting observations of these studies, what is clear is that there is no single R_{Dark}-leaf N relationship. What is less clear, however, is what role soil N and P play in determining rates of metabolism and relationships linking *A* and *R* to other leaf traits.

H~~As noted above, some studies assessing impact of nutrient availability on trait-trait relationships have used linear relationships fitted to untransformed data, while others have log transformed data on both axes. (e.g. Atkin *et al.*, 2013)~~[KC1]ow might deficiencies in N and/or P affect log-log relationships fitted to multi-species data sets~~made up of contrastingacross~~? When grown on limiting N supply, leaf N concentration can decrease to a greater extent than does the rate of metabolism, underpinned by an increase in the proportion of N allocated to metabolic processes (Makino *et al.*, 1994; Pons *et al.*, 1994; Cheng & Fuchigami, 2000); in such cases, low N treatment would result in higher rates of *A* per unit leaf N (i.e. higher ~~*PNUE*~~A_N). If the proportional

increase in A_N is similar among species, then only the proportionality-coefficient of log-log *A*-N plots would change (see Supporting Information Notes S1 for further details). However, if proportional changes in A_N are greater in some species than others ~~, although may A_{tN}~~ (e.g. Warren *et al.*, 2003; Pons & Westbeek, 2004; Hikosaka, 2010), then limitations in N supply might alter the scaling-exponent of log-log *A*-N relationships. ~~(Warren et al. 2003, (Pons & Westbeek, 2004; Hikosaka, 2010)For example, Warren *et al.* (2003) found that the maximal rate of carboxylation by Rubisco (V_{cmax}) per unit leaf N of *Pinus sylvestris* remained constant irrespective of N supply; others have reported similar observations (Pons & Westbeek, 2004; Hikosaka, 2010). Thus, it is uncertain how limitations in N supply affect the slope and/or elevation of log-log trait-trait relationships for data sets that include a wide range of species differing in leaf structure/chemistry and life history characteristics~~Similar issues are at play for how low P supply impacts on log-log *A*-N relationships, depending on: (1) the extent to which leaf phosphorus can be re-allocated from non-metabolic to metabolic pools (Woodrow *et al.*, 1984; Foyer & Spencer, 1986; Raghothama, 1999); (2) whether low P supply impacts on N allocation within leaves (Whitehead *et al.*, 2005; Reich *et al.*, 2009); and (3) the extent to which these responses differ among species ~~representative of~~on the leaf economic spectrum (Wright *et al.*, 2004).

Leaf respiration takes place both in the light (R_{Light}) and the dark, with R_{Light} typically lower than R_{Dark} (Way *et al.*, 2015), even when accounting for re-fixation of respiratory CO_2 (Pärnik *et al.*, 2007). Failure to account for light-induced inhibition of leaf *R* can lead to overestimation of ecosystem respiration (Wehr *et al.*, 2016). The extent of inhibition is highly variable, ranging from 80% inhibition through to cases where R_{Light} is 30% higher than R_{Dark} (Heskel *et al.*, 2014; Way & Yamori, 2014). While the mechanisms underpinning light inhibition are not fully understood, photorespiratory metabolism is likely involved (Randall *et al.*, 1990; Tcherkez *et al.*, 2008). Although the impact of nutrient gradients on light inhibition has been examined in a few field studies (Atkin *et al.*, 2013; Heskel *et al.*, 2013), little if any attention has been given to the main and interactive effects of N and P deficiency on the degree of light inhibition, or how N and P supply affect scaling relationships linking leaf R_{Light} to related traits. In addition, no study has yet addressed the question of whether there are systematic differences in light inhibition among species or plant functional types (PFTs). PFTs are used in terrestrial biosphere models (TBMs) as the starting point for predicting metabolic rates across the globe, with TBM-dependent foliar N often being the starting point for predicting rates of *A* and *R* (Clark *et al.*, 2011; Oleson *et al.*, 2013).

Given the importance of leaf trait relationships for the functioning of individual plants and whole ecosystems~~,~~ with strong tendencies for leaf N and P to co-vary in nature (Sterner & Elser, 2002; Reich & Oleksyn, 2004), it is difficult to isolate the effects of low P from low N in explaining broad patterns of variation in relationships between metabolism and leaf nutrients. This is especially so in field studies comparing ~~species from~~ among biomes, and among sites within individual biomes (Kattge *et al.*, 2009; Reich *et al.*, 2009; Domingues *et al.*, 2015). Given these challenges, we used a factorial design using 37 species from six PFTs to isolate the individual and combined effects of N and P ~~availability~~supply on a range of leaf traits associated with photosynthesis and respiration. Our study provides the first multi-PFT comparison assessing nutrient impacts on leaf trait relationships under controlled environment conditions. ~~and the fact that nutrient availability varies markedly in nature, it is vital that the impacts of nutrient supply *per se* on leaf trait-trait relationships be characterised. Past studies assessing impacts of N and P availability on trait-trait relationships have been field-based and, as such, not able to definitively assess the effects of nutrient supply on leaves. It was with this in mind that we conducted a controlled environment study using 37 species from six PFTs, with each species grown on high and low N and P supply.~~ We tested the following hypotheses:

1. ~~-~~Growth under low P supply will reduce average rates of leaf *A* and *R*, with the inhibitory effect being greater in high N than low N-grown plants~~, but have limited minimal effect in plants grown on~~when N is ~~limiting limiting~~N, reflecting Liebig's law of the minimum~~. Similarly, the deleterious effect of low N supply on *A* and *R* will be less pronounced when P is limiting. Such effects are expected to be similar among PFTs. ~~Similarly, the deleterious effect of low N supply on *A* and *R* will be less pronounced when P is limiting.~~
2. Irrespective of P supply, limitations in N supply will ~~the elevation (i.e. *y*-axis intercept) of log-log *A*-N, R_{Dark}-N and R_{Light}-N relationships will be higher when plants are grown on limiting N supply (Fig. 1).W~~increase the proportionality-coefficient of log-log *A*-N, R_{Dark}-N and R_{Light}-N relationships, but not alter the scaling-exponent (Fig. 1). ~~-limiting conditions~~
3. ~~Irrespective of N supply,~~ P deficiency will ~~the slope of~~ reduce the proportionality-coefficient of log-log *A*-N, R_{Dark}-N and R_{Light}-N relationships, with the proportional decline in metabolic rates at any given N being greater on high N supply than under low N supply ~~will be lower when plants are grown on limiting P supply~~ (Fig. 1).

4. *R*:*A* ratios will be greater for nutrient-limited plants ~~for~~ compared to plants grown on high N and P supply, reflecting the greater inhibitory effect of limiting nutrient supply on photosynthesis compared to that of respiration (Evans & Terashima, 1988).
5. ~~(Shapiro *et al.*, 2004; Tcherkez *et al.*, 2008; Ayub *et al.*, 2011; Crous *et al.*, 2012; Griffin & Turnbull, 2013)~~Nutrient treatments that reduce rates of photorespiration (i.e. limitations in N and/or P supply) ~~willmay~~will result in lower ~~There is no systematic difference in the~~ degrees of light inhibition of leaf *R* ~~among species or nutrient treatments~~ (see SI Notes S2 for further details).~~.~~

Materials and Methods

Plant material and experimental design

The study comprised 20 woody and 17 herbaceous species from 17 families, representing six PFTs: broadleaved trees (~~BLT~~BLT), broadleaved shrubs (S), coniferous needle-leaved trees (~~NLT~~NLT), C_3 grasses (C3G), C_3 herbs (C3H) and C_4 grasses (C4G) (Table S1). Four Protaceae species which can access P more efficiently under low P (Lambers *et al.*, 2010) were included in the woody species group (Table S1). The woody species were planted in November 2008, and measured starting in January 2009, whereas the herbaceous species were planted in November 2009 and measured in January 2010 onwards. Woody plants were sourced mainly from the Wagga Wagga Forestry Nursery (Wagga Wagga, NSW, Australia) whereas the herbaceous species were sourced from the Woodbridge nursery (Woodbridge, Tasmania) or grown from seeds (Nindethana Australian Seeds, Albany, WA, Australia). Twenty-four plants of each species were potted in sterilised sand in 50 cm tall PVC, 9 cm diameter, 3.18 L pipes to achieve six replicates per nutrient treatment. Two adjacent glasshouses were used with three replicate blocks in each glasshouse. Species and nutrient treatments were randomly allocated within each block. Species were grown in the glasshouse under natural light conditions at 25°C/18°C (day and night).

Nutrient treatments represented four combinations of different N and P concentrations: High N–High P (H_NH_P), High N–Low P (H_NL_P), Low N–High P (L_NH_P) and Low N–Low P (L_NL_P). The H_NH_P solution was based on Hoagland No. 1 solution (Hoagland & Arnon, 1950) with the following macronutrient composition: 1 mM KH_2PO_4, 5 mM KNO_3, 0.07 mM $CaCl_2$, 0.45 mM $MgSO_4.7H_2O$. The

~~Whereas the final concentrations of 'high N' solutions were 5 mM KNO_3, ~~'low N' solutions had a ~~final~~ concentration of 0.4 mM KNO_3 (modified after Atkinson *et al.*, 2007). The 'high P' solutions contained 1 mM KH_2PO_4 (Edwards et al., 2006) whereas 'low P' had 2.0 μM KH_2PO_4 to limit storage of P in the vacuole as a buffer (after Campbell & Sage, 2006). Thus, N:P supply ratios varied from 5:1 for H_NH_P to 2500:1 for H_NL_P, 0.4:1 for L_NH_P and finally 200:1 for L_NL_P. Here, our aim was to achieve changes in foliar chemistry ~~and function,~~that was also reflected in rates of leaf~~plant~~ metabolism, rather than mimic naturally occurring N:P ratios~~ as such rather than mimic natural soil N:P ratios~~. Each nutrient solution was balanced for cations and contained the same amount of micronutrients and FeEDTA. Micronutrients were constant in all solutions: 4.2 μM B, 1.2 μM Mn, 0.8 μM Zn, 0.03 μM Cu, 0.04 μM Mo and 0.01 μM Co. Iron was added as ferric EDTA to a level of about 8 μM Fe. These micronutrient concentrations were one-tenth of the recommended Hoagland solution because full strength has resulted in toxic symptoms (Leggett, 1971). Nutrient solutions were made up in 200 L black storage tubs, which were refilled regularly. Once per day, nutrient solutions were pumped out of 200 L tubs and delivered to the pots using irrigation tubing and drippers at 20 ml per minute. Plants received on average about 120-150 mL solution each day and were watered at the end of each day.

Gas exchange measurements

Gas exchange was measured between 9 am and 2:30 pm with portable infrared gas analysers (LiCor 6400, LiCor Inc., NE, USA). 873 light-response curves were conducted ~~on~~on the most recently fully developed leaves in each treatment~~ at a single timepoint~~, starting with light-saturating conditions (irradiance: 1800 μmol m^{-2} s^{-1}; flow: 500 μmol s^{-1}; leaf temperature: 25°C; reference CO_2: 400 μmol mol^{-1}). After these initial conditions, light was decreased to 1500 μmol m^{-2} s^{-1} and then 100 μmol m^{-2} s^{-1}, and finally in steps of 5-10 μmol m^{-2} s^{-1} to achieve estimates of respiration during the day (R_{Light}) using the Kok method (Kok, 1948). R_{Dark} was obtained 10 minutes after the light was turned off (flow 300 μmol s^{-1}) to avoid post-illumination transients (Atkin *et al.*, 1998). For each leaf, measurements were made at a single time point.

The Kok effect refers to the break in the slope of plots of net CO_2 uptake vs irradiance near the light-compensation point (Ayub *et al.*, 2011). Using this approach, a regression line is extrapolated from the linear part of the light-response curve (over the irradiance range 20-60 μmol m^{-2} s^{-1}), with the *y*-axis intercept representing R_{Light}. R_{Light} was corrected for changes in C_i (intercellular CO_2

concentration) associated with changes in irradiance (Kirschbaum & Farquhar, 1987). All curves were analysed using R (R Core Development Team, 2015).

Structural and chemical leaf composition

Leaves were collected after each light response curve and the leaf area inside the leaf cuvette was measured before drying at 70°C for at least 24 h to determine leaf mass per area (M_a). Total N and P were determined via Kjeldahl digestion (Allen, 1974), with digests analysed using a flow injection analyser (Lachat instruments, Loveland, Colorado, USA) for N and P using the indophenol blue (Scheiner, 1976) and ammonium molybdate methods (John, 1970) respectively. Leaves from 16 woody species were analysed for soluble sugars and starch according to Loveys *et al*. (2003).

Statistical analyses

Initial data exploration revealed cases of outlying (but not erroneous) values, unequal variation among treatment classes and non-normal distributions. Many of our variables, notably leaf N and P, showed distributions that were heavily right-skewed and therefore . For consistency of presentation and interpretation all variables have been log-transformed in the analyses that follow. Log transformation was the best option chosen from a number of alternative methods (e.g. square root and inverse) and thatwith improvement applied both to the underlying variables and to model residuals[KC2]. Because most variables followed a rightly skewed distribution, values were log-transformed before screening for outliers and subsequent analysis, as outlined in SI SM1. Data were filtered for outliers identified as those falling beyond twice the interquartile range with for a given variable. Ffilters for separate variables were applied to the dataset in the following order: P_m, N_m, M_a, $R_{Dark,m}$, and A_m. Application of these filters resulted in a total of 15 outliers/rows being removed from the dataset. Whenever an outlier was identified, the entire observational row was removed. Application of these filters resulted in a total of 15 outliers being removed from the dataset. After filtering, 37 species, six PFTs, and four nutrient treatments remained (Table S1) totalling 873 measurements (with 148 PFT species by treatment combinations). Woody species represented 472 observations. Further filtering was necessary for R_{Light}, removing three species (*Banksia spinulosa, Pinus halepensis and Banksia integrifolia*) due to inadequate replication or measurements with negative R_{Light} values (i.e. positive *y*-axis interceptselevation from Kok estimates – see abovemethod). Data falling beyond twice the interquartile range resulted in further removal of

~~twelve rows. Hence the R_{Light} dataset consisted of 710 values.~~ Calculations of means and ANOVA tests were conducted in JMP (SAS Institute, NC). We used individual plant data when comparing bivariate relationships across the six PFTs and four nutrient treatments, consistent with the rationale used in past studies (Reich *et al.*, 2008; Xiang *et al.*, 2013). Standardized major axis (SMA) analysis was used to determine the best-fitting lines (α = 0.05) for the key log-log trait-trait relationships (Falster *et al.*, 2006; Warton *et al.*, 2006; Warton *et al.*, 2012) ~~and leaf traits~~. We tested for differences among nutrient ~~supply~~ treatments and PFT classes. ~~SMA, descriptive statistics and filtering~~Data exploration, screening for outliers and SMA analysis was performed using the R language ~~and environment were done in R~~ (R Development Core Team, 2015) and the smatr package (Warton *et al*. 2012). Multiple comparison tests employed in the smatr package are explained in Appendix D of Warton *et al.* (2006) – see SI Notes S1 for further details. –Data are ~~to be~~ freely available and published on the Australian Research Data portal (~~-~~doi to be inserted~~:~~ here).

Results

Leaf trait variation

Across the entire dataset, there was large variation in each of the selected structural, chemical composition and physiology traits, reflecting the diverse range of species (Fig. ~~s~~ 2 ~~and 3~~). For all traits, there were significant differences among PFTs (P < 0.0001, Table 1). When averaged across all treatments, mean M_a ~~for each~~across PFTs varied five-fold, with needle-leaf coniferous (NLT) trees having exhibited~~ing~~ the highest values (225 ± 10 g m^{-2}) while C_4 ~~-~~grasses (C4G) showed the lowest M_a (39 ± 2 g m^{-2}) (Fig. 2~~a~~c, Table S2). ~~This pattern was also reflected in N_a (3.6 ± 0.3 g m^{-2} in NlT and 0.7 ± 0.02 g m^{-2} in C4G) and P_a (0.93 ± 0.13 g m^{-2} in NLT) with the average of broadleaved shrubs and trees (BLT) being 0.37 ± 0.05 g m^{-2} while grasses exhibited mean P_a values of 0.18 g m^{-2} (C3G) and 0.13 g m^{-2} (C4G) (Fig. 2b). Overall, woody species exhibited higher N_a than their herbaceous counterparts, largely reflecting differences in M_a among PFTs and PFT-dependent differences in N_m (Table S2). Highest P_a were again found in NlT (0.93 ± 0.13 g m^{-2}) with the average of broadleaved shrubs and trees being 0.37 ± 0.05 g m^{-2}, while grasses exhibited mean P_a values of 0.18 g m^{-2} (C3G) and 0.13 g m^{-2} (C4G)(Fig. 2c).~~ N:P ratios showed significant differences between each treatment (Table 2) with N:P above 15 in broad-leaved trees (BLT), shrubs (S) and C_3 grasses (C3G) ~~; PFT differences are shown in~~ (Table S2). Mass-based values of both nitrogen (N_m) and

phosphorus (P_m) were lowest in S and NLT and highest in C4G and C3H (Table S2, Fig. 2). ~~Within each PFT, high and low nutrient treatment concentrations were reflected in the leaf traits summarised in Table S2 and Figure S1.~~ Mean A_a values were similar across PFTs, ranging from 10.1 to 15.3 μmol m^{-2} s^{-1} for C3G and S, respectively (Fig. ~~3a~~2d); A_m was highest in C4G and C3H and lowest for ~~NLT~~NLT (data not shown). Highest rates of $R_{Dark,a}$ were found for S and ~~BLT~~BLT (mean 1.13 μmol m^{-2} s^{-1}) with the lowest means in C3G (0.49 μmol m^{-2} s^{-1}) (Fig. ~~3b~~2e). By contrast, $R_{Light,a}$ was highest in ~~NLT~~NLT and C4G (mean 0.95 μmol m^{-2} s^{-1}) and lowest in C3G (0.44 μmol m^{-2} s^{-1}) (Fig. ~~3c~~2f, Table S2).

Impacts of nutrient supply on mean values

Table S2 shows mean values for each PFT both among and across treatments, while Table 1 shows the two-way ANOVA assessing main and interactive effects of PFT and treatment. Growth of plants on contrasting N and P supply resulted in significant changes in foliar N and P, both on a mass and area basis (Table 1). Although there were differences in leaf chemistry among PFTs (see above and Table S2), overall treatments receiving high N supply exhibited the highest N_m (24.5 mg g^{-1}, averaged across both H_NH_P and H_NL_P; Table 2) compared to the low N treatments with an average N_m of 15.9 mg g^{-1}. Low P treatment reduced N_m and N_a with high N supply, whereas under low N supply, P supply did not impact on leaf N (Table 2, Fig. S1). The highest P_m were found in high P supply both for high N and low N conditions, with the lowest P_m found in high N-low P supply (H_NL_P). Low P supply resulted in a decline in P_a on both high and low N supply. Interestingly, low N-grown plants exhibited higher P_m compared to high N-grown plants (Table 2, Fig. S1). For woody PFTs (i.e. ~~BLT~~BLT, ~~NLT~~NLT and S), there was no effect of low N on area-based soluble sugars (Table 3). Under high N conditions, low P supply led to a 60% increase in starch and a 15% increase in total non-structural carbohydrates (TNC) per unit leaf area in the woody PFTs (Table 3 and SI Table S3). Whereas N and P supply had large effects on foliar chemistry, nutrient supply had no effect on M_a, averaged across all PFTs (Tables 1 and 2, Fig. S1a). Moreover, correcting M_a values for accumulation of TNC did not alter this conclusion, when considering woody species alone. The stability of M_a suggests we can equally assess the effect of nutrient supply on metabolic traits on either an area- or mass-basis.

Nutrient supply had a marked effect on fluxes of area- and mass-based rates of photosynthesis and respiration (Tables 1 and 2). The highest rates of both *A* and *R* occurred in the

H_NH_P treatment and all other treatments exhibited similar, lower rates of A_a and A_m. ~~-~~ A_a and A_m were reduced to a similar extent when either N or P were in low supply (reduced by 20% for A_a) and with no further reduction when both N and P were in low supply (Table 2). Similarly, for R_{Dark} (both on area and mass bases) low N treatment resulted in significant declines (-27%), as did low P supply (-16%) compared to the H_NH_P treatment. However, unlike photosynthesis, L_NL_P supply resulted in further significant declines in R_{Dark} compared to when either N or P were in low supply (Table 2). R_{Light} showed similar patterns as R_{Dark}, with lowest rates in L_NL_P. Compared to the H_NH_P treatment, R_{Light} exhibited reduced rates in low N regardless of P supply (Table 2). Hence, there was no effect of P deficiency on both photosynthesis and respiration (in either the dark or light) in low N-grown plants (both L_NH_P and L_NL_P), with P deficiency only affecting fluxes in high N-grown plants (Table 2).

When averaged across all PFTs, ~~P~~photosynthetic N use efficiency (A_N) was 11% enhanced when plants experienced low N supply (compared to H_NH_P). By contrast, ~~A_N was reduced by~~ low P supply only reduced average A_N in plants grown on high N (-20%) ~~but not when N was limiting~~ (Table 2). For respiration, N and P deficiencies had little effect on N-based rates of R_{Dark} and R_{Light} (Table 2). For both A and R, rates expressed on a foliar P basis were significantly higher in low P-grown plants, irrespective of N supply (Table 2), suggesting greater P-use efficiency under low P conditions.

N-deficiency had no effect on respiration:photosynthesis ratios (i.e. the inhibitory effect of low N on A and R was similar) when P was non-limiting (Table 2). By contrast, under P-deficient conditions, low N supply resulted in a slight decrease in the R_{Dark}:A and R_{Light}:A ratios, reflecting the significant decline in respiration alone (i.e. no change in A) under L_NL_P compared to H_NL_P conditions (Table 2). Finally, nutrient availability had no effect on the R_{Light}:R_{Dark} ratio (Table 2), with the average the R_{Light}:R_{Dark} ratio among treatments being 0.74 μmol m^{-2} s^{-1} (n = 710). Thus, light inhibited leaf respiration by 26% across the entire dataset.

Table 1 also provided insights into whether the effect of N supply on each trait differed among PFTs. The absence of a significant interaction term indicated that all PFTs were similarly affected by availability of N and P for M_a, A_a and $R_{Light,a}$ (Table 1). For $R_{Dark,a}$, the effect of treatment did differ among PFTs (Table S2); in general, low N supply reduced R in ~~BIT~~BLT and S but not in ~~NIT~~NLT or grasses. Growth on H_NL_P supply led to significantly lower $R_{Dark,a}$ in ~~BIT~~BLT alone, with no effect of P supply on other PFTs (Table S2). At low N, low P supply only reduced rates of $R_{Dark,a}$ in C3G. Taken together, these results point to a general pattern of low N supply having its greatest

impact on metabolic traits when P is non-limiting (~~thus~~ supporting Hypothesis 1) and that the impact of nutrient supply is more commonly seen in broadleaved trees and shrubs than the other PFTs.

Leaf trait relationships

The above analyses point to increased rates of A_N under low N supply (irrespective of P supply), and reduced A_N when P is limiting (for high N plants only), suggesting nutrient-mediated shifts in relationships between photosynthesis and N. We explored whether such changes were associated with shifts in proportionality-coefficient (i.e. elevation of the log-log relationships) ~~elevation~~ and/or scaling-exponents (i.e. slope) ~~slopes~~ of the A_a-N_a relationship (as hypothesized in Fig. 1) and other area-based trait relationships via standardized major axis (SMA) analyses.

Figure ~~4~~ 3 shows bivariate relationships among N_a, P_a and M_a, with the corresponding SMA results shown in Table 4. While substantial scatter occurred within each bivariate relationship, significant positive relationships were apparent for each separate nutrient combination. For the N_a-M_a relationship, there were no significant differences in scaling-exponents ~~slopes~~ of the four treatments, but significant differences were found in the proportionality-coefficient ~~elevation~~ of the fitted lines (Fig. ~~4a~~3a, Table 4). Here, N_a at a given M_a was significantly higher in the H_NH_P treatment, followed by H_NL_P, which in turn was higher than L_NH_P and L_NL_P relationships (i.e. H_NH_P > H_NL_P > L_NH_P = L_NL_P). Thus, leaves consistently accumulated more leaf N for a given M_a under high N conditions, with P deficiency slightly decreasing how much N accumulated at given M_a. For the P_a-M_a relationship (Fig. 3b), ~~there was a significant difference in slopes among the treatments because~~ L_NH_P had a ~~steeper~~ greater scaling-exponent ~~slope~~ compared to the other treatments (Table 4); Figure ~~4b~~ 3b also shows that P_a values at a given M_a were lower in the two low-P treatments than the two high-P treatments. When considering the N_a-P_a relationship, SMA analyses revealed differen~~tces among the~~ scaling-exponents~~slopes~~, with L_NH_P exhibiting a flatter N_a-P_a response than the other treatments. N_a at a given P_a was highest in H_NL_P plants (Fig. ~~4c~~3c), followed by L_NL_P and H_NH_P grown plants, which in turn were markedly higher than L_NH_P plants. Thus, the treatments differed in a manner consistent with N:P values (Table 2).

We now explore how the treatments affected bivariate relationships linking A_a to N_a, P_a and M_a (Fig. ~~5~~4, Table 4). For A_a-N_a relationships (Fig. ~~5a~~4a), ~~showed different slopes. Ssimilar~~ growth on low N supply resulted in greater scaling-exponents ~~slopes~~ compared to high N grown plants,

irrespective of P supply (Table 4). ~~This finding reflected two interesting outcomes. Firstly, for~~For plants grown on high P supply, growth on low N (L_NH_P) only increased *A* at a given N (i.e. increased A_N) in species with inherently high N_a values, with little effect of N supply on A_N in low N_a species. By contrast, when P supply was limiting, low N supply had little effect on A_N in high N_a species, but increased A_N in low N_a species. Thus, the effect of N supply on A_N differs among the contrasting species used in our study, and depends on whether P supply was limiting. ~~were exhibited by the two low N treatments (i.e. L_NH_P & L_NL_P) but plants grown on H_NL_P exhibited a much steeper slope than the other treatments. Hence~~Underpinning this observation was that ~~, with high N availability, growth on~~ low P supply reduced A_N in low N_a species, but had little effect on A_N in high N_a species.

~~had a marked inhibitory effect on photosynthesis, but when N was limiting, P availability had no effect.~~ For A_a-P_a relationships (Fig. ~~5b~~4b), H_NL_P grown plants exhibited a significantly ~~steeper~~ greater scaling-exponent ~~slope~~ than the other treatments (Table 4), with A_a at a given P_a differing between high and low N treatments. For the low P-grown plants, those provided with high N exhibited generally greater A_a at a given P_a than low N-grown plants (i.e. comparing green with ~~yellow~~orange~~, and blue with red black lines~~ symbols in Fig. ~~5b~~4b; note: A_a-P_a relationship was not significant for L_NL_P plants). Finally, when considering the relationship between A_a and M_a, no relationship was found for high N-grown plants (Table 4), with a common relationship being shared by low N grown (L_NH_P and L_NL_P) plants (grey line in Fig. 4c). Taken together, photosynthesis was reduced with low P supply in high N-grown plants with respect to the A_a-N_a relationship, whereas ~~but~~ with low N supply ~~leading to reduced~~ rates of A_a for a given P_a were reduced~~,~~ both in high and low P availability.

For relationships linking leaf respiration to ~~other traits~~ N_a, P_a and ~~mass:area~~M_a, we found that treatment had no effect on the scaling-exponents ~~slopes~~ of $R_{Dark,a}$-N_a, but did alter the scaling-exponents ~~slopes~~ of the $R_{Light,a}$-N_a relationship (Fig. ~~65~~4, Table 4). For $R_{Dark,a}$-N_a, plants grown on high N, P-deficiency resulted in lower $R_{Dark,a}$ at a given N_a (i.e. lower proportionality-coefficient)~~,~~ ~~particularly in plants where foliar N concentrations were low~~. By contrast, at low N supply, availability of P had no effect on $R_{Dark,a}$ at a given N_a (Fig. ~~6a5~~4~~a~~d); this was also true for the $R_{Light,a}$-N_a relationship (Fig. ~~6d5~~4~~d~~g). Thus, as was the case for photosynthesis, P availability only modifies the relationship between leaf respiration and N_a when N supply is not limiting.

Growth on low P resulted~~s~~ ~~exhibited~~ in higher $R_{Dark,a}$ and $R_{Light,a}$ at a given P_a, irrespective of N supply (Fig. ~~6b5~~4e~~b~~, ~~e~~h). Moreover, limitations in the availability of N resulted in lower $R_{Dark,a}$ and

$R_{Light,a}$ at a given P_a; this was true for plants grown on low and high P. Thus, relationships linking leaf respiration to P_a ~~are~~ were influenced not only by the availability of P, but also by N supply. Finally, whereas no significant relationships were found when considering $R_{Light,a}$ as a function of M_a (Fig. ~~6f5~~4~~f~~i, Table 4), $R_{Dark,a}$-M_a relationships were significant for all four treatments (albeit with a low R^2; Fig. ~~6c5~~4f~~c~~, Table 4). Interestingly, expressing rates on a mass basis resulted in highly significant $R_{Light,}$~~$_a$~~$_m$-M_a (and $R_{Dark,}$~~$_a$~~$_m$-M_a) relationships (SI Fig. S2, Table S4). For $R_{Dark,a}$-M_a (Fig. 4f), nutrient treatment had no effect on relationship slopes, with elevations being ranked from highest to lowest: $H_NH_P > H_NL_P = L_NH_P > L_NL_P$. Thus, the general pattern was one of $R_{Dark,a}$ at a given M_a decreasing as nutrient availability declined (Table 2).

Nutrient availability did not significantly affect rates of $R_{Dark,a}$ at a given A_a indicated via a common $R_{Dark,a}$-A_a relationship across all four treatments (shown by the common grey regression line in Fig. ~~7a6~~5a). ~~-~~While there were significant differences in scaling-exponents ~~slopes~~ when considering $R_{Light,a}$-A_a (Fig. ~~7b6~~5b, Table 4), the overall pattern was similar to that seen in $R_{Dark,a}$-A_a ~~Figure 7a~~. On first inspection, the finding that $R_{Dark,a}$-A_a was unaffected by nutrient supply contrasts with the comparison of means shown in Table 2, where limitations in N supply reduced $R_{Dark,a}$:A_a ratios in low P-grown plants. However, given the scattered nature of the bivariate plots and the slight differences in $R_{Dark,a}$ at a given A_a among the treatments (Fig. ~~7~~6~~5~~a), caution is needed when considering small changes in mean-alone data shown in Table 2. Thus, the most parsimonious conclusion is that nutrient supply had little or no effect on $R_{Dark,a}$:A_a ratios. This was also true for the $R_{Light,a}$-$R_{Dark,a}$ relationships among four treatments ~~, with nutrient supply having no effect~~ (Fig. ~~9~~6~~5c~~a; Table ~~4~~4); consequently, the degree of light inhibition was unaffected by nutrient supply (~ 26% inhibition; Table 2).

While ~~nutrient supply had no significant effect on the slope in Figure 7a~~6a~~, there remained considerable scatter in the overall $R_{Dark,a}$-A_a relationship. Thus, other factors distinct from nutrient supply must be responsible for the scatter. Differences between PFTs~~ woody and herbaceous species showed ~~is an obvious candidate via~~ no significant difference in ~~slopes~~scaling-exponents (P = 0.37) for R-A relationships, w~~ but significant differences in elevation (Table S5). W~~woody species exhibited~~itinged~~ a higher proportionality-coefficient ~~elevation (i.e. less negative y-axis intercept)~~ compared to herbaceous PFTs (Fig. ~~S3a~~6~~5~~d, Table ~~S5~~4). Hence, for a given A_a, woody species overall had higher dark respiration compared to herbaceous species, with $R_{Dark,a}$ at a given A_a being highest in shrubs and broadleaf trees followed by C_3 herbs, while ~~needle-leaf~~NLT ~~trees~~, C_3 and C_4 grasses

exhibited the lowest $R_{Dark,a}$ at a given A_a (Fig. S3, Table S5). For $R_{Light,a}$-A_a (Fig. 5e), there was no difference between herbs and woody species in the scaling-exponent ~~slope~~ or proportionality-coefficient ~~elevation of the $R_{Light,a}$-A_a relationship~~ (but there was a shift moving along the same relationship); this is ~~in~~ contrast to $R_{Dark,a}$-A_a (Fig. ~~6~~5~~e~~d) where woody species exhibited a higher proportionality-coefficient than herbs. For the R_{Light}-R_{Dark} relationship, h~~H~~erbaceous species ~~showed~~exhibited a ~~higher~~greater scaling-exponent ~~slope in the R_{Light}-R_{Dark} relationship~~ compared to woody species (Fig. 5~~6~~f, Table ~~S~~5). ~~with t~~The lowest degree of light inhibition of leaf respiration was found in C4G (as shown by the highest R_{Light}:R_{Dark} slope, Table S5) and the highest degree of light inhibition found in NLT (lowest R_{Light}:R_{Dark} scaling-exponent~~slope~~).

~~, with $R_{Dark,a}$ at a given A_a being highest in shrubs and broadleaf trees followed by C_3 herbs, with needle-leaf trees, C_3 and C_4 grasses exhibiting the lowest $R_{Dark,a}$ at a given A_a (Fig. 8, Table 5). SMA analyses on woody and herbaceous PFTs indicated no significant difference in slopes (P = 0.37) but different elevations, with woody species exhibiting a higher elevation (i.e. less negative y-axis intercept) compared to herbaceous PFTs (Fig. S3a, Table 5). Hence, for a given A_a, woody species had higher dark respiration compared to herbaceous species.~~

~~For $R_{Light,a}$-A_a, there was less consistency in the bivariate relationships among PFTs (Table 5). When combining PFTs into woody and herbaceous categories, then there was no difference in slope or intercept of the $R_{Light,a}$-A_a relationship (but there was a shift moving along the same relationship) in contrast to $R_{Dark,a}$-A_a (Fig. S3b). Lastly, Figure 9 shows $R_{Light,a}$-$R_{Dark,a}$ relationships among four treatments, with nutrient supply having no effect (Fig. 9a; Table 4); consequently, the degree of light inhibition was unaffected by nutrient supply (~ 26% inhibition; Table 2). However, the lowest degree of light inhibition found in C4G (highest R_{Light}:R_{Dark} slope, Table 5) and the highest degree of light inhibition found in NlTNLT (lowest R_{Light}:R_{Dark} slope). Combining PFT into woody and herbaceous species indicated a higher R_{Light}:R_{Dark} slope in herbaceous compared to woody species (Fig. 9b, Table 5).~~

Discussion

~~*Study rationale and* ~~*Eextent of nutrient limitations*

~~Nitrogen and phosphorus exert strong and fundamental controls on metabolic performance of leaves, as seen across both natural and fertilized soil gradients (Reich & Schoettle, 1988; Cordell *et al.*, 2001; Whitehead *et al.*, 2005). However, given strong tendencies for leaf N and P to co-vary in nature (Sterner & Elser, 2002; Reich & Oleksyn, 2004; Ågren *et al.*, 2012), it is difficult to isolate the effects of low P from low N in explaining broad patterns of variation in relationships between metabolism and leaf nutrients. This is especially so in field studies comparing species from among biomes and among sites within individual biomes (Kattge *et al.*, 2009; Reich *et al.*, 2009; Domingues *et al.*, 2015; Bahar *et al.*, 2016). Given these challenges, we used a factorial design to isolate the individual and combined effects of N and P availability on a range of leaf traits associated with photosynthesis and respiration. Our findings show that both N- and P-supply can mediate leaf trait-trait relationships, suggesting that the next generation of Earth System Models should consider how limitations in N *and* P supply in individual biomes may affect trait relationships (Goll *et al.*, 2012).~~

In setting up our experiment, there was a risk that the low P and N treatments would not result in P- and N-deficient phenotypes (in terms of lower metabolic rates) needed to test our working hypotheses. ~~-~~Past studies have shown that total P concentrations are strongly influenced by storage of P in vacuoles (Foyer & Spencer, 1986; Raghothama, 1999) and this storage pool can buffer cytosolic P. ~~, h~~Hence measured differences in ~~foliar~~leaf P may not show~~ing a~~ metabolically different phenotypes ~~despite measured differences in foliar P~~. Similarly, the fact that investment of leaf N in metabolism is a plastic trait – for example, increasing as a proportion of total N when N supply is limiting (Cheng & Fuchigami, 2000) - means that low N treatments will not~~on't~~ necessarily result in lower metabolic rates. However, we found rates of A_a and R_a that were lower in low P-grown plants (compared to H_NH_P), demonstrating that the supply of P was sufficiently low to create P-deficient phenotypes (Table 2). Moreover, as reported previously ~~for a range of plant species~~ (Hammond & White, 2008), starch accumulation occurred in low P-grown plants of the woody PFTs (Table 3), consistent with a P-~~-~~deficient phenotype. The low N treatment also resulted in reductions in A_a and R_a, and reduced mass-based concentrations of soluble sugars in woody PFTs (Table 3), demonstrating that N-deficient phenotypes were achieved. Importantly, the impact of the low P-

and N treatments on metabolic rates and ~~area-based~~log-log trait-trait relationships depended on the extent to which supply of each nutrient was limiting. ~~Our study provides the first multi-PFT comparison assessing nutrient impacts on leaf trait relationships under controlled environment conditions.~~

How does P mediate A-R-*N relationships, and is the impact of P independent of N supply?*

~~Past work has shown that rates of *A* at a given N are often lower at sites with low P availability (Meir *et al.*, 2002; Kattge *et al.*, 2009; Reich *et al.*, 2009; Domingues *et al.*, 2010; Bahar *et al.*, 2016).~~ Based on knowledge of how P deficiencies affect leaf metabolism (Warren & Adams, 2002; Plaxton & Tran, 2011; Bloomfield *et al.*, 2014; Ellsworth *et al.*, 2015) and studies comparing trait relationships of plants growing on soils with contrasting P levels (Kattge *et al.*, 2009; Reich *et al.*, 2009), we predicted that P deficiency would reduce the proportionality-coefficient of log-log *A*-N, R_{Dark}-N and R_{Light}-N relationships, but with no change in the scaling-exponent of the log-log relationships ~~shallower *A*-N slopes in P-limited plants~~ (Fig. 1). However, ~~contrary to the hypothesized relationships outlined in Fig. 1, inspection of~~ Figure 4a shows that ~~growth on limiting P supply did not result in a *decreased* slope in the A_a-N_a relationship; rather,~~ when plants were grown on high N, low P resulted in a significant increase in scaling-exponent ~~slope~~ of A_a-N_a relationship, reflecting a decrease in A_a in low N_a species with low, with P deficiency having little or no effect on A_a in species with high N_a. The same was true for the $R_{Dark,a}$-N_a relationship (Fig. 4d)~~,~~ ~~consistent with Rowland *et al.* (2016) reporting higher rates of R_{Dark} for a given N in low nutrient soils~~. While the reasons for this increase in scaling-exponent under low P-high N conditions remain uncertain, the results highlight how contrasting species differing in leaf structure/chemistry differ in their responses to P deficiency~~Stimulation~~[KC3] ~~of A_a (or $R_{Dark, a}$) under low P conditions (when N is not limiting but not under N-limiting conditions) might reflect the fact that N supply can play a role in stimulating P uptake in low P soils (Treseder & Vitousek, 2001)~~.

~~One of our working hypotheses was that the effect of~~We expected that low P supply on metabolic rates would be greatest in plants supplied with high N, based on past observations on the stimulatory effect of P addition being greatest when N is non-limiting (e.g. Aerts *et al.*, 1992; Meir *et al.*, 2001). ~~We found~~Our results support that ~~l~~low P supply had minimal effects on metabolic rates when N was limiting (Table 2) - both for photosynthesis and respiration - while the inhibitory effect of low P was greatest when N was not limiting. ~~These results are consistent with Liebig's law~~

~~where the most limiting element has the greatest impact.~~ A ~~phosphorus~~P ~~supply~~-mediating effect on metabolic relationships with N has been reported in several tropical studies (Reich & Walters, 1994; Denton *et al.*, 2007; Meir *et al.*, 2007; Mercado *et al.*, 2011; Quesada *et al.*, 2012), which typically have lower P availability compared to N supply reflected in high N:P ratios.

Interestingly, P had *no* effect at all on the log-log A_m-N_m relationship irrespective of N supply (Fig. S2). ~~-~~The latter result contrasts with Reich *et al.* (2009), where log-log A_m-N_m relationships differed among plants growing at P-rich and P-deficient sites from field studies representing a range of nutrient availability, soil properties and mycorrhizal associations, thermal environment, water availability and species composition. In controlled-~~-~~environment conditions~~our study~~, we compared the effect of low P supply on a common set of species ~~grown under common, controlled environment conditions~~, suggesting that contrasting relationships (via different ~~slopes~~ scaling-exponents or ~~intercepts~~ proportionality-coefficients~~elevation~~) from different sites and biomes may include variation other than just soil nutrient supply. Given this, care is needed when interpreting the direct role of nutrient availability on A_m-N_m relationship slopes across field sites.

Mechanisms underpinning changes in photosynthetic N use efficiency.

Consistent with our working hypotheses (Fig. 1), we found that overall mean values of leaf-level N-use efficiency of photosynthesis (A_N) ~~was~~ were enhanced by low N supply, both under low and high P supply (Table 2~~, Fig. 5a4a~~). Cheng & Fuchigami (2000) found that A_N of apple leaves also increased with decreasing N supply. However, not all studies have reported this response, with Warren *et al.* (2003), Hikosaka (2010) and Pons & Westbeek (2004) all reporting little effect of N supply on photosynthetic N use efficiency. Our data suggest that while low N treatment increased A_N of some species – particularly species that exhibit inherently low N_m values (Fig. S2) – low N treatment had little or no effect on other species (e.g. high N_m species). Given that species with inherently low N_m tend to also be slow-growing and/or found in less productive environments (Wright *et al.*, 2004), this suggests that plasticity of A_N in response to variation in N supply may systematically vary among species adapted to contrasting environments. The increase in A_N in low N supply can occur via several possible ways (Hikosaka, 2004), such as: (1) greater fraction of leaf N allocated to photosynthetic processes, particularly the relative investment of leaf N in Rubisco (Evans & Seeman, 1989; Poorter & Evans, 1998; Warren *et al.*, 2003); (2) increase in the activation state of Rubisco (Stitt & Schulze, 1994; Warren *et al.*, 2000); (3) reduction of N investment in

589 defence compounds, cell walls and/or nucleic acids (Chapin & Kedrowski, 1983; Onada *et al.*, 2004;
590 Harrison *et al.*, 2009); or (4) a decrease in inorganic N accumulation (e.g. NO_3^- in vacuoles) to
591 maintain organic N investment in metabolism. Similar factors are also likely to play a role in
592 decreased A_N exhibited by high N-grown plants with limiting P supply (Table 2) (Warren & Adams,
593 2002; Bloomfield *et al.*, 2014). Indeed, there was a negative relationship in all PFTs between N-use
594 efficiency and N:P ratio with more P-limited plants (high N:P ratio) exhibiting lower nitrogen-use
595 efficiencies for both A_N and $R_{Dark,N}$ (Fig. S4).
596
597 *Plant functional types and bivariate relationships*
598 In our study, 37 species from six PFTs were used ~~to create a spectrum of leaf traits varying in~~
599 ~~structure, chemistry and function, which enabled us~~ to assess whether the effect of nutrient supply
600 on leaf traits differed among the PFTs. ~~Table 1 showed that for all leaf chemistry traits, t~~The
601 magnitude of nutrient-mediated changes in leaf traits differed among the PFTs (Table 1). ~~-~~Proteoid
602 species exhibited steeper A_a-N_a relationships when P was low compared to broadleaved trees but
603 not when P was high (Fig. S5). However, the distinct PFTs exhibited relatively similar responses to
604 treatments when considering structural (i.e. M_a) and metabolic traits (particularly A_a and $R_{Light,a}$).
605 Thus, despite enormous differences in leaf life span, leaf nutrients and structural components
606 among the PFTs, generally speaking all PFTs exhibited similar metabolic responses to nutrient
607 availability, consistent with previous field studies (Reich *et al.*, 2008).
608 ~~While n~~Nutrient supply had no effect on the ratio between leaf dark respiration and
609 photosynthesis (i.e. ~~no significant difference in slope~~a common relationship across all four
610 treatments in Fig. 5a). However, ~~our finding of~~there was PFT-dependent variation in $R_{Dark,a}$-A_a
611 scaling (Fig. ~~8~~5d~~6~~, Fig. S3), with PFTs differing in the proportional-coefficients of relationships that
612 shared common scaling-exponents. This contrasts with recent field-based comparisons of PFTs in
613 respiration-photosynthesis relationships (Atkin *et al.*, 2015), where there was little evidence of C_3
614 herbs/grasses, broad leaved trees, shrubs and needle-leaved trees differing in rates of $R_{Dark,a}$ at a
615 given A_a. However, such comparisons may be strongly influenced by a range of environmental
616 factors that may mask underlying PFT-dependent patterns. Our study, conducted under controlled
617 environment conditions provided strong evidence of PFT-mediated variations in the respiration:
618 photosynthesis ratio in plants driven by differences between woody and herbaceous species.
619 Future work will be needed to determine what underlying factors (e.g. respiratory costs associated

with protein turnover, phloem loading and maintenance of ion gradients in leaf cells; Lambers, 1985; Noguchi & Yoshida, 2008) are responsible for this PFT-dependent variation in $R_{Dark,a}$-A_a scaling.

R_{Light} versus R_{Dark}

One of the objectives of our study was to assess whether nutrient supply affected the degree of light inhibition of non-photorespiratory mitochondrial CO_2 release (see SI Notes S2 for further details). Past work has reported that R_{Light} is often lower than R_{Dark}, with the degree of inhibition being highly variable (Griffin & Heskel, 2013) but typically around 30% (Crous *et al.*, 2012; Weerasinghe *et al.*, 2014). In leaves, respiratory energy is needed for assimilation of N and protein turnover, as well as for supporting the ATP demands from photosynthesis (Krömer, 1995). Given that N deficiency may limit such demands for respiratory products, one possibility was that the degree of inhibition could be linked to nutrient supply, with low N potentially affecting factors such as demand for TCA cycle intermediates and photorespiratory capacity. In our study, we found that ~~(Ayub *et al.*, 2014)However, as shown in Fig. 9b~~6c, ~~-~~across a broad range of species, nutrient supply had no effect on the relationship between R_{Dark} and R_{Light} (Fig. 5c), with the overall degree of light inhibition being 26% across all treatments and species. Interestingly, the degree of light inhibition was generally less in herbaceous species compared to woody species (Fig. ~~9b~~6f), a result that merits testing in a broader set of species.

Conclusions

~~Our study assessed the impact of P limitations both under high and low N conditions, with the results leading to the following insights: Our findings show that both N- and P-supply can mediate leaf trait-trait relationships, suggesting that the next generation of Earth System Models should consider how limitations in N *and* P supply~~availability ~~in individual biomes may affect trait relationships (Goll *et al.*, 2012).~~

~~Linking plant metabolism to leaf nutrients, both A-N and R-N relationships were only affected by low P when N availability was high. Low P supply did not have an effect on the slope of *A*-N and *R*-N relationships when N was limiting. Thus, P availability only modified the relationship between leaf *R* and N when N supply was not limiting (high N).~~ Our findings do not support the hypothesized responses shown in Figure 1; rather, rates of leaf *A* and *R* at a given leaf N were dependent on P

supply in species with inherently low N_a values but not in those with inherently high N_a values. Further work assessing patterns of N and P allocation will be needed to address what factors are responsible for these observations. Interestingly, our results also did not support Hypothesis 4, with ~~This finding supports our H.2 and is consistent with results from field-based studies (especially in tropical ecosystems with high N:P ratios). Whereas less P is needed to meet metabolic demands in N-deficient plants, in high N grown plants, the effect of low P supply may be underpinned by insufficient P allocated to metabolic processes or the extent to which P supply affect N allocation in the plant.~~ ~~[KC4]~~

~~In *A*-P and *R*-P relationships, low P reduced *A* and *R* for a given P_a compared to high P, supporting the first part of H.1 and H.3, but low N supply reduced *A* and *R* rates *both* in high and low P availability. In contrast to H.4,Photosynthesis rates were reduced under low P supply in high N-grown plants in *A*-N and *R*-N relationships. By contrast, P supply had no effect at a given N_a when N supply was limiting. Low N supply led to reduced *A* and *R*, both in high and low P supply. Thus, P availability only modified the relationship between leaf *R* and N when N supply was not limiting (high N).~~

~~N~~nutrient supply ~~had~~ having no effect on the ratio between R_{Dark} and A_a~~, although woody species had a higher respiration rates for a given *A* compared to herbaceous species~~. ~~This~~The fact that coupling between respiration and photosynthesis remained constant suggests that deficiencies in nutrient availability have similar inhibitory effects on the capacity of processes. ~~suggests that respiration proportionally declined with photosynthesis across species and nutrient treatments.~~ ~~Lastly~~Further, light inhibition of respiration was constant across nutrient treatments (26% on average) – again not supporting our working hypothesis - but with lower degrees of light inhibition in herbaceous than in woody species. Both findings have relevance to ecosystem models seeking to predict future rates of carbon exchange, as the degree of light inhibition can strongly influence ecosystem respiration estimates (Wohlfahrt *et al.*, 2005; Wehr *et al.*, 2016). Finally, the fact that both N- and P-supply can mediate leaf trait-trait relationships suggests that the next generation of Earth System Models may need to consider how limitations in N and P availability affect trait-trait relationships used to predict large scale carbon fluxes.

~~Nutrient supply had no effect on the ratio between R_{Light} and R_{Dark}, with a light inhibition across treatments of 26%. Interestingly, our results point to a lower degree of light inhibition in herbaceous than in woody species.~~

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Acknowledgements

The support of the Australian Research Council (ARC) to O.K.A. (FT0991448, DP0986823, DP1093759, DP130101252 and CE140100008) and P.M. (FT110100457) is acknowledged, and P.M. acknowledges the support of UK NERC (NE/C51621X/1, NE/F002149/1). KYC is grateful for support from the ARC as a DECRA ~~research~~ fellow (DE160101484). We thank Steph McCaffery and David Sherlock for their expert technical assistance.

Author contributions

O.K.A., K.L.G., M.H.T., P.M. and K.Y.C. designed the study. K.Y.C., O.S.S., J.Z. and A.C.A.N. collected the gas exchange data while O.S.S. ~~also~~ conducted leaf nutrient analyses. K.Y.C. led data processing, analyses and writing with substantial contributions from O.K.A. and K.J.B.. All authors made conceptual contributions throughout the manuscript development.

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The following Supporting Information is available for this article:

Notes S1 Analysing trait-trait relationships

Notes S2 Light inhibition of leaf respiration to photorespiratory metabolism: Hypothesis 5

~~**Supplementary Figures and Tables:**~~

~~**Table S1** Species averages ± standard error of the mean per nutrient treatment for leaf mass per area ratio (M_a), area based leaf nitrogen (N_a) and leaf phosphorus (P_a), light saturated photosynthesis (A_a), leaf respiration in darkness ($R_{Dark,a}$) and in the light ($R_{Light,a}$).~~

~~**Table S2** Means ± standard errors for several leaf (nitrogen (N), phosphorus (P) and leaf mass per area ratio, M_a) and metabolic traits (light-saturated photosynthesis (A_a), leaf respiration in darkness ($R_{Dark,a}$) and in the light ($R_{Light,a}$) on either an area- or mass basis (indicated with a or m subscripts respectively) for each functional group and nutrient treatment.~~

~~**Table S3** Means ± standard error of mass-based total soluble sugars, starch and TNC (total non-structural carbohydrates) on 16 woody species for each nutrient treatment.~~

~~**Table S4** Standardized Major Axis regression slopes and their confidence intervals for mass-based log transformed bivariate relationships in each nutrient treatment.~~

~~**Table S5** Standardized Major Axis regression slopes and their confidence intervals for area-based log transformed bivariate relationships in each PFT or between herbaceous and woody PFT groups.~~

Fig. S1 Box plots showing the variation of leaf structural and chemical traits for four different nutrient treatment combination of nitrogen (N) and phosphorus (P) supply~~: H_NH_P (high N – high P), H_NL_P (High N – Low P), L_NH_P (Low N – High P) and L_NL_P (Low N – Low P). Traits shown are: (a) leaf mass per unit leaf area (M_a); (b) area-based leaf nitrogen concentration (N_a); and (c) area-based leaf phosphorus concentration (P_a).~~

Fig. S2 ~~R~~Log-log relationships between ~~(a, d)~~ mass-based light-saturated photosynthesis ~~(A_a)~~, ~~(b, e) mass-based~~ dark respiration ~~($R_{Dark,a}$)~~ and ~~(c, f) mass-based~~ mitochondrial respiration in the light

($R_{Light,a}$) as a function mass-based leaf nitrogen concentrations (N_m) (upper panels) and as a function of leaf mass per unit leaf area (M_a) (lower panels). SMA analyses were done on individual observations within each nutrient combination treatment: H_NH_P (high N – high P, red), H_NL_P (High N – Low P, green), L_NH_P (Low N – High P, blue) and L_NL_P (Low N – Low P, yellow).

Fig. S3 Log-log relationships between area-based dark respiration ($R_{Dark,a}$) and light-saturated photosynthesis showing individual observations within each plant functional type across all nutrient treatments: C4G, grass with C4 photosynthetic pathway; C3G, grass species with C3 photosynthetic pathway; C3H, C3 metabolism herb; S, shrub; BLT, broad-leaved tree; NLT, needle-leaved tree. All values are shown on a $\log_{10}$ scale. See Table S5 for standardized major axis (SMA) regression outputs.

Log-log relationships between (a) area-based dark respiration ($R_{Dark,a}$) and light-saturated photosynthesis and (b) area-based mitochondrial respiration in the light ($R_{Light,a}$) showing individual observations within herbaceous (herb) and woody species. See Table 5 for standardized major axis (SMA) regression outputs.

Fig. S4 Log-log relationships between (a) N-based dark respiration ($R_{Dark,N}$) and (b) photosynthetic nitrogen use efficiency (A_N) as a function of N:P ratio for six plant functional types.

Fig. S5 Log-log relationships of nitrogen-use efficiency (via photosynthesis-N relationships) between proteoid broadleaved species (yellow) and Broadleaved trees (BlT, blue) in each nutrient treatment: H_NH_P (high N – high P, H_NL_P (High N – Low P), L_NH_P (Low N – High P) and L_NL_P (Low N – Low P). Nitrogen-use efficiency was significantly ($P < 0.05$) higher for Proteoids when grown in low P conditions (right panels) but not when P supply was high (left panels).

Table S1 Species averages per nutrient treatment for leaf mass per area ratio (M_a), area-based leaf nitrogen and leaf phosphorus, light-saturated photosynthesis, leaf respiration in darkness and in the light.

Table S2 Means for several leaf structural/chemical composition and metabolic traits on either an area- or mass basis.

Table S3 Means of mass-based total soluble sugars, starch and total non-structural carbohydrates on 16 woody species for each nutrient treatment.

Table S4 Standardized Major Axis regression slopes and their confidence intervals for mass-based log transformed bivariate relationships in each nutrient treatment.

Table S5 Standardized Major Axis regression slopes and their confidence intervals for area-based log transformed bivariate relationships in each PFT or between herbaceous and woody PFT groups.

Table 1 Two-way ANOVA testing for differences between PFT and Nutrient treatments (Treatm).

	M_a		N_m		P_m		N_a		P_a		A_a		$R_{Dark,a}$		$R_{Light,a}$	
Source	DF	F	DF	F	DF	F	DF	F	DF	F	DF	F	DF	F	DF	F
PFT	5	204^{***}	5	42.8^{***}	5	43.7^{***}	5	142^{***}	5	38.8^{***}	5	12.4^{***}	5	50.7^{***}	5	10.8^{***}
Treatm	3	1.9^{ns}	3	83.2^{***}	3	253^{***}	3	78.9^{***}	3	181^{***}	3	7.2^{***}	3	8.1^{***}	3	3.5^{*}
PFT x Treatm	15	1.4^{ns}	15	2.4^{**}	15	8.3^{***}	15	2.38^{**}	15	6.3^{***}	15	1.1^{ns}	15	1.9^{*}	15	0.34^{ns}
~~Error~~ Within	777		779		779		768		768		744		735		566	
Total	800	45.7^{***}	802	23.4^{***}	802	58.4^{***}	791	43.9^{***}	791	42.9^{***}	767	4.7^{***}	758	14.1^{***}	589	4.1^{***}

Variables shown are: leaf mass per unit area (M_a), mass-based leaf nitrogen (N_m) and leaf phosphorus (P_m), area-based leaf nitrogen values (N_a) and phosphorus (Pa), area-based rates of light-saturated photosynthesis (A_a) and area-based rates of leaf respiration in darkness ($R_{Dark,a}$) and in the light ($R_{Light,a}$). Degrees of freedom (DF) and F-ratio (F) are indicated for each variable with statistical significance indicated as follows: *** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$, ns is non-significant. All models were highly significant with error and total degrees of freedom varying depending on the variable.

Table 2 Means and standard error for leaf traits and metabolic fluxes measured on individual plants across ~~plant~~PFTs ~~functional groups~~.

		Treatment			
Trait	Units	High N High P	High N Low P	Low N High P	Low N Low P
N_m	mg g^{-1}	26.69 ± 0.85^{a}	22.21 ± 0.64^{b}	16.42 ± 0.65^{c}	15.31 ± 0.55^{c}
P_m	mg g^{-1}	3.88 ± 0.20^{a}	1.07 ± 0.09^{b}	5.52 ± 0.30^{c}	1.69 ± 0.12^{d}
N:P	dimensionless	8.57 ± 0.29^{a}	34.56 ± 1.55^{b}	4.72 ± 0.26^{c}	15.00 ± 0.87^{d}
M_A	g m^{-2}	113.18 ± 7.22^{a}	118.82 ± 5.77^{a}	109.75 ± 5.71^{a}	119.96 ± 6.16^{a}
A_a	µmol m^{-2} s^{-1}	15.59 ± 0.48^{a}	13.01 ± 0.48^{b}	12.17 ± 0.43^{b}	12.13 ± 0.41^{b}
A_m	nmol g^{-1} s^{-1}	245.42 ± 17.77^{a}	168.02 ± 12.56^{b}	162.00 ± 9.83^{b}	149.59 ± 8.15^{b}
A_N	µmol g_{N} ~~N~~ s^{-1}	8.65 ± 0.41^{a}	6.92 ± 0.39^{b}	9.56 ± 0.36^{c}	9.55 ± 0.36^{c}
A_P	µmol g_{P} ~~P~~ s^{-1}	72.99 ± 3.80^{a}	195.35 ± 8.98^{b}	43.84 ± 2.72^{c}	131.42 ± 6.90^{d}
$R_{Dark,a}$	µmol m^{-2} s^{-1}	1.07 ± 0.05^{a}	0.90 ± 0.04^{b}	0.78 ± 0.04^{bc}	0.74 ± 0.03^{c}
$R_{Dark,m}$	nmol g^{-1} s^{-1}	14.67 ± 0.87^{a}	10.68 ± 0.66^{b}	9.94 ± 0.61^{b}	8.79 ± 0.51^{c}
$R_{Dark,N}$	µmol g_{N} ~~N~~ s^{-1}	0.56 ± 0.03^{b}	0.47 ± 0.03^{a}	0.60 ± 0.03^{c}	0.57 ± 0.03^{bc}
$R_{Dark,P}$	µmol g_{P} ~~P~~ s^{-1}	4.87 ± 0.30^{a}	14.24 ± 0.87^{b}	2.50 ± 0.15^{c}	7.62 ± 0.45^{d}
$R_{Light,a}$	µmol m^{-2} s^{-1}	0.86 ± 0.05^{a}	0.76 ± 0.05^{ab}	0.67 ± 0.05^{bc}	0.61 ± 0.05^{c}
$R_{Light,m}$	nmol g^{-1} s^{-1}	11.83 ± 1.12^{a}	9.41 ± 1.06^{b}	9.18 ± 1.10^{b}	8.31 ± 1.14^{b}
$R_{Light,N}$	µmol g_{N} ~~N~~ s^{-1}	0.43 ± 0.03^{a}	0.39 ± 0.03^{a}	0.52 ± 0.06^{a}	0.49 ± 0.06^{a}
$R_{Light,P}$	µmol g_{P} ~~P~~ s^{-1}	3.77 ± 0.30^{c}	11.31 ± 0.91^{a}	2.01 ± 0.05^{d}	6.10 ± 0.53^{b}
R_{Dark}:A	dimensionless	0.076 ± 0.004^{ab}	0.086 ± 0.007^{a}	0.070 ± 0.004^{ab}	0.064 ± 0.002^{b}
R_{Light}:A		0.056 ± 0.004^{ab}	0.061 ± 0.006^{a}	0.052 ± 0.005^{bc}	0.046 ± 0.004^{c}
R_{Light}:R_{Dark}		0.701 ± 0.03^{a}	0.787 ± 0.06^{a}	0.741 ± 0.04^{a}	0.731 ± 0.05^{a}

The sample size for each leaf trait varied between 217-220 (171-183 for R_{Light} variables). Leaf traits presented here are mass-based nitrogen (N_m) and phosphorus (P_m) concentrations, their ratio (N:P) and leaf mass per area ratio (M_A) Metabolic traits are light saturated photosynthesis (A) and dark respiration (R_{Dark}), presented on an area-, mass, N- and P-basis as well as the R/A ratio. Lowercase letters indicated significant differences between different nutrient treatments at $P < 0.05$ in a least square means Student-t post-hoc test.

995 **Table 3 Means and standard error of the mean across Plant Functional Type (PFT) for soluble sugars,**
996 **starch and non-structural carbohydrates (TNC)** both on a mass-basis (upper panel) and an area-basis
997 (bottom panel), including sample size (n).

Treatment	Units	H_NH_P	H_NL_P	L_NH_P	L_NL_P
n		217	218	218	220
MASS-BASED					
Soluble Sugars	mg g^{-1}	51.85 ± 3.35^{a}	43.13 ± 3.62^{ab}	39.34 ± 2.76^{b}	39.81 ± 2.68^{b}
Starch	mg g^{-1}	35.20 ± 4.57^{b}	56.74 ± 7.87^{a}	41.99 ± 7.27^{b}	62.41 ± 10.21^{ab}
TNC	mg g^{-1}	87.05 ± 6.53^{a}	99.88 ± 8.84^{a}	81.32 ± 8.21^{a}	102.22 ± 10.44^{a}
AREA-BASED					
Soluble Sugars	g m^{-2}	7.44 ± 1.07^{a}	6.20 ± 0.68^{a}	5.29 ±0.56^{a}	6.20 ± 0.57^{a}
Starch	g m^{-2}	3.80 ± 0.44^{b}	6.89 ± 1.08^{a}	5.12 ± 0.99^{b}	8.52 ± 1.46^{a}
TNC	g m^{-2}	11.24 ± 1.32^{bc}	13.09 ± 1.26^{ab}	10.41 ± 1.19^{c}	14.72 ± 1.53^{a}
M_a (TNC_{corr})	g m^{-2}	137.7 ± 16.2^{a}	130.6 ± 7.8^{a}	128.0 ± 11.4^{a}	143.8 ± 11.2^{a}

998

999 Different letters indicate statistical differences between treatments at $P < 0.05$ in a Least square means
1000 Student-t post-hoc test after log transformation. Also shown are M_a corrected for area-based TNC (TNC_{corr}).
1001 Nutrient treatments are defined as follows: High N–High P (H_NH_P), High N–Low P (H_NL_P), Low N–High P
1002 (L_NH_P) and Low N–Low P (L_NL_P).

Table 4 Standardized Major Axis coefficients for area-based log-transformed bivariate relationships for Figs 3, 4 and 5.

						Starting model				Simpler model, common slopes					
Fig.	Model	Treat.	*n*	R^2	*p*-value	Scaling exponent (β_1) (±CIs)	Proportionality coefficient (β_0) (±CIs)	H0: no diff. in β_1 (*p*-value)	Pairwise comp.	Common β_1 (±CIs)	Proportionality coefficient (β_0) (±CIs)	H0: no diff. in β_0 (*p*-value)	Pairwise comp.	Common β_0 (±CIs)	H0: no diff. in axis shift (*p*-value)
3a	N_a - M_a	H_NH_P	182	0.675	< 0.0001	0.901 (0.829, 0.98)	-1.424 (-1.571, -1.277)	0.4955		0.854 (0.814, 0.897)	-1.334 (-1.417, -1.251)	< 0.0001	A		< 0.0001
		H_NL_P	177	0.603	< 0.0001	0.838 (0.763, 0.921)	-1.356 (-1.514, -1.198)				-1.388 (-1.473, -1.302)		B		
		L_NH_P	186	0.443	< 0.0001	0.835 (0.749, 0.93)	-1.518 (-1.697, -1.339)				-1.557 (-1.642, -1.471)		C		
		L_NL_P	184	0.466	< 0.0001	0.822 (0.739, 0.915)	-1.513 (-1.689, -1.337)				-1.577 (-1.663, -1.49)		C		
3b	P_a - M_a	H_NH_P	182	0.475	< 0.0001	1.053 (0.947, 1.171)	-2.608 (-2.827, -2.39)	< 0.0001	A						
		H_NL_P	177	0.025	0.0361	0.95 (0.821, 1.101)	-3.034 (-3.316, -2.752)		A						
		L_NH_P	186	0.369	< 0.0001	1.463 (1.304, 1.642)	-3.288 (-3.623, -2.953)		B						
		L_NL_P	184	0.147	< 0.0001	1.143 (0.999, 1.308)	-3.21 (-3.52, -2.899)		A						
3c	N_a - P_a	H_NH_P	182	0.624	< 0.0001	0.856 (0.782, 0.936)	0.808 (0.756, 0.861)	< 0.0001	A						
		H_NL_P	177	0.057	0.0014	0.882 (0.764, 1.019)	1.321 (1.167, 1.474)		A						
		L_NH_P	186	0.265	< 0.0001	0.57 (0.504, 0.646)	0.358 (0.312, 0.404)		B						
		L_NL_P	184	0.119	< 0.0001	0.719 (0.627, 0.825)	0.795 (0.693, 0.898)		A						
4a	A_a - N_a	H_NH_P	182	0.094	< 0.0001	0.747 (0.649, 0.858)	0.926 (0.878, 0.975)	0.0008	C						
		H_NL_P	177	0.070	0.0004	1.135 (0.983, 1.31)	0.702 (0.633, 0.77)		A						
		L_NH_P	186	0.250	< 0.0001	0.921 (0.813, 1.045)	0.929 (0.894, 0.963)		B						
		L_NL_P	184	0.211	< 0.0001	0.895 (0.786, 1.019)	0.935 (0.899, 0.97)		B,C						
4b	A_a - P_a	H_NH_P	182	0.026	0.0306	0.639 (0.553, 0.738)	1.53 (1.462, 1.598)	< 0.0001	B						
		H_NL_P	177	0.049	0.0030	1.001 (0.866, 1.157)	2.2 (2.026, 2.375)		A						
		L_NH_P	186	0.057	0.0010	0.526 (0.457, 0.605)	1.258 (1.207, 1.309)		B						
		L_NL_P	184	0.017	0.0745										
4c	A_a - M_a	H_NH_P	183	0.001	0.6419										
		H_NL_P	177	0.003	0.4969										
		L_NH_P	189	0.023	0.0379	0.77 (0.668, 0.887)	-0.469 (-0.687, -0.251)	0.9540		0.767 (0.694, 0.848)	-0.464 (-0.621, -0.308)	0.4150		-0.475 (-0.629, -0.321)	0.5442
		L_NL_P	190	0.020	0.0505	0.765 (0.664, 0.882)	-0.485 (-0.705, -0.264)				-0.489 (-0.648, -0.33)				
4d	$R_{dark,a}$ - N_a	H_NH_P	182	0.140	< 0.0001	1.03 (0.899, 1.18)	-0.363 (-0.427, -0.299)	0.2024		1.142 (1.068, 1.221)	-0.397 (-0.453, -0.341)	< 0.0001	B		< 0.0001
		H_NL_P	177	0.080	0.0001	1.271 (1.102, 1.466)	-0.508 (-0.585, -0.432)				-0.469 (-0.524, -0.414)		C		
		L_NH_P	186	0.223	< 0.0001	1.121 (0.987, 1.274)	-0.296 (-0.34, -0.253)				-0.299 (-0.34, -0.257)		A		
		L_NL_P	184	0.188	< 0.0001	1.171 (1.027, 1.336)	-0.347 (-0.394, -0.299)				-0.343 (-0.388, -0.299)		A,B		
4e	$R_{dark,a}$ - P_a	H_NH_P	182	0.049	0.0027	0.881 (0.764, 1.017)	0.47 (0.378, 0.561)	< 0.0001	B						
		H_NL_P	177	0.035	0.0127	1.121 (0.969, 1.298)	1.17 (0.973, 1.367)		A						
		L_NH_P	186	0.208	< 0.0001	0.64 (0.562, 0.728)	0.105 (0.051, 0.159)		C						
		L_NL_P	184	0.054	0.0015	0.842 (0.731, 0.971)	0.585 (0.46, 0.71)		B						
4f	$R_{dark,a}$ - M_a	H_NH_P	183	0.074	0.0002	0.929 (0.807, 1.069)	-1.834 (-2.091, -1.577)	0.5230		0.969 (0.903, 1.04)	-1.912 (-2.053, -1.77)	< 0.0001	A		0.0563
		H_NL_P	177	0.045	0.0047	1.066 (0.922, 1.232)	-2.232 (-2.544, -1.919)				-2.041 (-2.186, -1.895)		B		
		L_NH_P	189	0.081	< 0.0001	0.938 (0.817, 1.076)	-2.002 (-2.259, -1.744)				-2.063 (-2.205, -1.922)		B		
		L_NL_P	190	0.047	0.0026	0.958 (0.833, 1.102)	-2.116 (-2.388, -1.844)				-2.137 (-2.283, -1.992)		C		

Table 4 contd.

						Starting model				Simpler model, common slopes					
Fig.	Model	Treat.	*n*	R^2	*p*-value	Scaling exponent (β_1) (±CIs)	Proportionality coefficient (β_0) (±CIs)	H0: no diff. in β_1 (*p*-value)	Pairwise comp.	Common β_1 (±CIs)	Proportionality coefficient (β_0) (±CIs)	H0: no diff. in β_0 (*p*-value)	Pairwise comp.	Common β_0 (±CIs)	H0: no diff. in axis shift (*p*-value)
4g	$R_{light,a}$ - N_a	H_NH_P	152	0.082	0.0004	1.299 (1.114, 1.515)	-0.629 (-0.726, -0.531)	0.0471	A						
		H_NL_P	138	0.041	0.0176	1.572 (1.332, 1.854)	-0.757 (-0.876, -0.639)		A,B						
		L_NH_P	148	0.044	0.0107	1.738 (1.482, 2.038)	-0.586 (-0.68, -0.492)		B						
		L_NL_P	134	0.040	0.0209	1.679 (1.42, 1.986)	-0.588 (-0.685, -0.492)		B						
4h	$R_{light,a}$ - P_a	H_NH_P	152	0.039	0.0150	1.082 (0.925, 1.267)	0.425 (0.3, 0.55)	0.0016	B,C						
		H_NL_P	138	0.053	0.0068	1.455 (1.235, 1.715)	1.399 (1.115, 1.683)		A						
		L_NH_P	148	0.109	< 0.0001	0.969 (0.831, 1.131)	0.06 (-0.04, 0.16)		C						
		L_NL_P	134	0.032	0.0397	1.317 (1.113, 1.559)	0.891 (0.658, 1.125)		A,B						
4i	$R_{light,a}$ - M_a	H_NH_P	152	0.024	0.0573					1.35 (1.244, 1.464)				-2.938 (-3.158, -2.718)	
		H_NL_P	138	0.019	0.1077										
		L_NH_P	151	0.003	0.4968										
		L_NL_P	138	0.000	0.8346										
5a	$R_{dark,a}$ - A_a	HNHP	187	0.128	< 0.0001	1.373 (1.2, 1.572)	-1.635 (-1.854, -1.416)	0.1637		1.234 (1.163, 1.311)	-1.476 (-1.573, -1.379)	0.0600		-1.45 (-1.532, -1.368)	< 0.0001
		HNLP	187	0.191	< 0.0001	1.109 (0.974, 1.263)	-1.284 (-1.441, -1.126)				-1.415 (-1.506, -1.324)				
		LNHP	191	0.387	< 0.0001	1.222 (1.093, 1.368)	-1.435 (-1.581, -1.29)				-1.448 (-1.532, -1.364)				
		LNLP	190	0.428	< 0.0001	1.253 (1.124, 1.397)	-1.509 (-1.654, -1.364)				-1.49 (-1.574, -1.406)				
5b	$R_{light,a}$ - A_a	HNHP	154	0.204	< 0.0001	1.75 (1.517, 2.017)	-2.228 (-2.524, -1.931)	0.0035	B,C						
		HNLP	145	0.094	0.0002	1.504 (1.286, 1.759)	-1.89 (-2.157, -1.623)		C						
		LNHP	151	0.190	< 0.0001	2.186 (1.89, 2.527)	-2.712 (-3.064, -2.359)		A						
		LNLP	138	0.191	< 0.0001	2.018 (1.734, 2.35)	-2.553 (-2.892, -2.214)		A,B						
5c	$R_{light,a}$ - $R_{dark,a}$	HNHP	154	0.525	< 0.0001	1.615 (1.447, 1.804)	-0.239 (-0.284, -0.195)	0.4906		1.613 (1.518, 1.714)	-0.239 (-0.284, -0.195)	0.0948		-0.188 (-0.216, -0.161)	< 0.0001
		HNLP	145	0.402	< 0.0001	1.536 (1.352, 1.744)	-0.181 (-0.236, -0.125)				-0.177 (-0.233, -0.121)				
		LNHP	151	0.425	< 0.0001	1.742 (1.541, 1.969)	-0.155 (-0.217, -0.093)				-0.169 (-0.225, -0.113)				
		LNLP	138	0.412	< 0.0001	1.554 (1.365, 1.769)	-0.165 (-0.231, -0.099)				-0.157 (-0.219, -0.094)				
5d	$R_{dark,a}$ - A_a	Herb	361	0.165	< 0.0001	1.122 (1.021, 1.234)	-1.408 (-1.521, -1.295)	0.4462		1.864 (1.663, 2.089)	-2.359 (-2.592, -2.127)	< 0.0001			< 0.0001
		Woody	394	0.354	< 0.0001	1.177 (1.087, 1.275)	-1.334 (-1.44, -1.227)				-2.253 (-2.459, -2.047)				
		Herb	-	-	-	1.154 (1.086, 1.227)	-1.441 (-1.519, -1.363)								
		Woody	-	-	-	1.154 (1.086, 1.227)	-1.308 (-1.39, -1.227)								
5e	$R_{light,a}$ - A_a	Herb	267	0.104	< 0.0001	1.864 (1.663, 2.089)	-2.359 (-2.592, -2.127)	0.5959		1.9 (1.715, 2.105)	-0.059 (-0.119, 0.001)	0.5008		-2.318 (-2.472, -2.165)	< 0.0001
		Woody	321	0.198	< 0.0001	1.789 (1.621, 1.975)	-2.253 (-2.459, -2.047)				-0.248 (-0.277, -0.219)				
5f	$R_{light,a}$ - $R_{dark,a}$	Herb	267	0.281	< 0.0001	1.9 (1.715, 2.105)	-0.059 (-0.119, 0.001)	0.0005							
		Woody	321	0.576	< 0.0001	1.521 (1.416, 1.634)	-0.248 (-0.277, -0.219)								

Coefficients of determination (R^2) and significance values (*P*-value) of each log-log trait-trait relationship are shown in addition to 95% confidence intervals (CI) of SMA slopes (scaling-exponent) and elevation (proportionality-coefficient). When scaling-exponents were significantly different between nutrient treatments ($P < 0.05$) in the starting model, a post hoc test showed pairwise comparisons between treatments, indicated with capital letters. When scaling-exponents were not significantly different, SMA tests for proportionality-coefficient differences were conducted using a common scaling-exponent (simpler model). Where there was a significant difference in proportionality-coefficient of the common scaling-exponent SMA regressions, values for the proportionality-coefficient are pairwise comparisons were indicated via different letters in post-hoc testing. When elevation using a common scaling-exponent showed no significant differences, a test investigating potential shifts along a relationship was conducted (last column) indicated via a *P*-value.

Figure Legends[OA5][OA6]

Fig. 1 Theoretical log-log relationships linking metabolic rates to leaf nitrogen con~~concentration~~tent among plant functional types. Changes associated with Hypothesis 2 and 3~~2~~ are illustrated and show expected changes in ~~slopes (scaling exponent) and/or y-axis intercepts~~elevation (~~proportionality coefficient~~proportionality-coefficient) of log-log relationships. Ellipses represent anticipated metabolic rate-N concentration combinations in each of the four treatments (high N-high P, high N-low P, low N-high P and low N-low P) used in our study, with their standardized major axes (SMAs) shown. Note that as a consequence of Hypothesis 1, we anticipate low P supply to reduce rates of metabolism when N is abundant, but ~~with~~ less so~~not~~ when N is limiting. When N is limiting, average rates of metabolism are predicted to be slightly lower ~~similar~~ in ~~high and~~ the low P-grown plants, resulting in ~~but with~~ a ~~shallower slope~~lower ~~scaling~~ proportionality-coefficient ~~exponent in the low P-grown plants~~of the SMA regressions compared to high-P grown plants,. Irrespective of P supply, we anticipate low N supply to result in an increase in ~~the y-axis intercepts~~elevation ~~of log-log relationships linking~~~~lower~~higher metabolic rates ~~to~~ for a given level of leaf N, reflecting increased relative allocation of leaf N to photosynthesis when N is limiting.

Fig. 2 Box plots showing the variation of leaf ~~structural and~~ chemical traits and leaf metabolism of carbon fluxes for six different plant functional type (PFT) classifications across all nutrient treatments. Traits shown are: (a) mass-based leaf nitrogen concentration (N_m); (b) mass-based leaf phosphorus concentration (P_m); (c) leaf mass per unit leaf area (M_a); ~~(b) area-based leaf nitrogen concentration (N_a); and (c) area-based leaf phosphorus concentration (P_a);~~ (d) area-based light-saturated photosynthesis (A_a); (e) area-based leaf mitochondrial respiration in darkness ($R_{Dark,a}$); and (f) area-based leaf mitochondrial respiration in the absence of photorespiration ($R_{Light,a}$).~~.~~ Data shown are for individual observations to give an indication of underlying data distribution. The boxes indicate the interquartile range and median. Observations outside the whiskers are shown as individual points. PFT classifications: C4G, grass species with C4 photosynthetic pathway; C3G, grass species with C3 photosynthetic pathway; C3H, C3 metabolism herb; S, shrub; ~~BlT~~BLT, broad-leaved tree; ~~NlT~~NLT, needle-leaved tree. Post-hoc differences among PFT are shown in Table S2 (across treatments column).

~~**Fig. 3** Box plots showing the variation of leaf metabolism of carbon fluxes for six different plant functional type (PFT) classifications. Traits shown are: (a) area-based light-saturated photosynthesis (*Aa*); (b) area-based leaf mitochondrial respiration in darkness ($R_{Dark,a}$); and (c) area-based leaf mitochondrial respiration in the absence of photorespiration ($R_{Light,a}$). Data shown are for individual observations to give an indication of underlying data distribution. PFT classifications: C4G, grass with C4 photosynthetic pathway; C3G, grass species with C3 photosynthetic pathway; C3H, C3 metabolism herb; S, shrub; BlTBLT, broad-leaved tree; NlTNLT, needle-leaved tree.~~

Fig. ~~4~~ 3 Relationships between area-based leaf nitrogen ~~levels~~ (N_a) and ~~area-based~~ leaf phosphorus ~~concentration~~ (P_a) concentration as a function of area-based leaf mass per unit leaf area (M_a) (panels (a) and (b) respectively) and the relationship between N_a versus P_a (panel (c)). All values are shown on a $\log_{10}$ scale. SMA analyses were done on individual observations within each nutrient combination treatment: H_NH_P (high N – high P, ~~red~~black), H_NL_P (High N – Low P, green), L_NH_P (Low N – High P, blue) and L_NL_P (Low N – Low P, ~~yellow~~orange). See Table 4 for standardized major axis (SMA) regression outputs.

Fig. ~~5~~ 4 Relationships between: (1) area-based rates of leaf CO_2 exchange ~~light-saturated photosynthesis (A_a)~~ as a function of (a, d and g) area-based leaf nitrogen concentration (N_a), (b, e, and h) area-based leaf phosphorus concentration (P_a), ~~and~~ (c, f and i) leaf mass per unit leaf area (M_a). Upper, middle and lower panels show light-saturated photosynthesis (A_a), area-based dark respiration ($R_{Dark,a}$) and mitochondrial respiration in the light ($R_{Light,a}$), respectively. ~~.~~ All values are shown on a $\log_{10}$ scale. SMA analyses were done on individual observations within each nutrient combination treatment: H_NH_P (high N – high P, ~~red~~black), H_NL_P (High N – Low P, green), L_NH_P (Low N – High P, blue) and L_NL_P (Low N – Low P, ~~yellow~~orange). See Table 4 for standardized major axis (SMA) regression outputs.

~~**Fig. 6 5** Relationships between area-based dark respiration ($R_{Dark,a}$) (upper panels) as a function of (a) area-based leaf nitrogen concentration (N_a), (b) area-based leaf phosphorus concentration (P_a) and (c) leaf mass per unit leaf area (M_a), while lower panels show mitochondrial respiration in the light ($R_{Light,a}$) as a function of (d) area-based leaf nitrogen concentration (N_a), (e) area-based leaf phosphorus concentration (Pa) and (f) leaf mass per unit leaf area (M_a). All values are shown on a~~

~~log_{10} scale. SMA analyses were done on individual observations within each nutrient combination treatment: H_NH_P (high N – high P, redblack), H_NL_P (High N – Low P, green), L_NH_P (Low N – High P, blue) and L_NL_P (Low N – Low P, yelloworange). See Table 4 for standardized major axis (SMA) regression outputs. Regression lines are not shown when relationships were not significant.~~

Fig. ~~7 6~~5 Log-log trait-trait r~~R~~elationships ~~on a log_{10} scale among nutrient treatments (upper panels)~~ between: (a) area-based dark respiration ($R_{Dark,a}$) and light-saturated photosynthesis (A_a) across nutrient treatments, with the single regression line showing the overall relationship, given the lack of slope differences among nutrient treatments (Table 4)[OA7]; ~~and~~ (b) area-based mitochondrial respiration in the light ($R_{Light,a}$) and A_a; and (c) area-based R_{Light} and area-based R_{Dark} showing SMA regression lines for each treatment.~~-~~ Values shown are for individual observations within each nutrient combination treatment: H_NH_P (high N – high P, black), H_NL_P (High N – Low P, green), L_NH_P (Low N – High P, blue) and L_NL_P (Low N – Low P, orange). See Table 4 for standardized major axis (SMA) regression outputs. ~~All values are shown on a log_{10} scale.~~ The bottom panels (d, e, f) reflect the same relationships for individual observations within herbaceous (herb, blue) and woody species (orange) with SMA outputs in Table S5. ~~In (a) and (b), values shown are for individual observations within each nutrient combination treatment: H_NH_P (high N – high P, red), H_NL_P (High N – Low P, green), L_NH_P (Low N – High P, blue) and L_NL_P (Low N – Low P, yellow). See Table 4 for standardized major axis (SMA) regression outputs.~~

~~**Fig. 8** Relationships between (a) area-based dark respiration ($R_{Dark,a}$) and light-saturated photosynthesis showing individual observations within each plant functional type across all nutrient treatments: *C4G*, grass with *C4* photosynthetic pathway; *C3G*, grass species with *C3* photosynthetic pathway; *C3H*, *C3* metabolism herb; S, shrub; BlTBLT, broad-leaved tree; NlTNLT, needle-leaved tree. All values are shown on a log_{10} scale. See Table 5 for standardized major axis (SMA) regression outputs.~~

~~**Fig. 9** Relationships between area-based mitochondrial respiration in the light ($R_{Light,a}$) and area-based dark respiration ($R_{Dark,a}$) showing individual observations within (a) each nutrient treatment being H_NH_P (high N – high P, red), H_NL_P (High N – Low P, green), L_NH_P (Low N – High P, blue) and L_NL_P (Low N – Low P, yellow) and (b) within woody and herbaceous plant species. All values are shown~~

~~on a log_{10} scale. See Table 4 for standardized major axis (SMA) regression outputs for each nutrient treatment and Table 5 for standardized major axis (SMA) regression outputs for woody versus herbaceous species~~

Figure 1

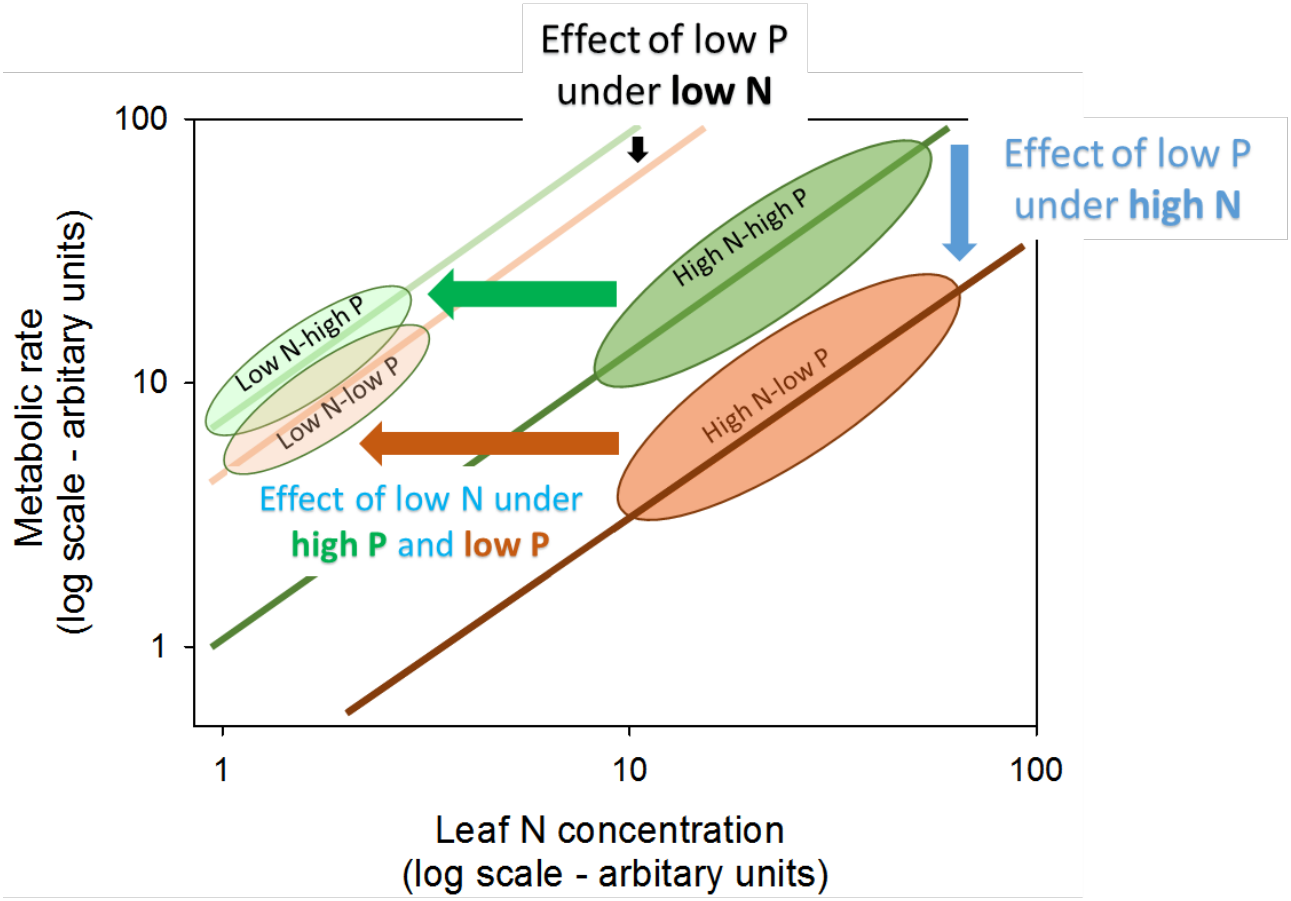

Effect of low P
under low N
Effect of low P
under high N
100
10
1
Metabolic rate
(log scale - arbitary units)
Low N-high P
Low N-low P
High N-high P
High N-low P
Effect of low N under
high P and low P
1
10
100
Leaf N concentration
(log scale - arbitary units)

Figure 2

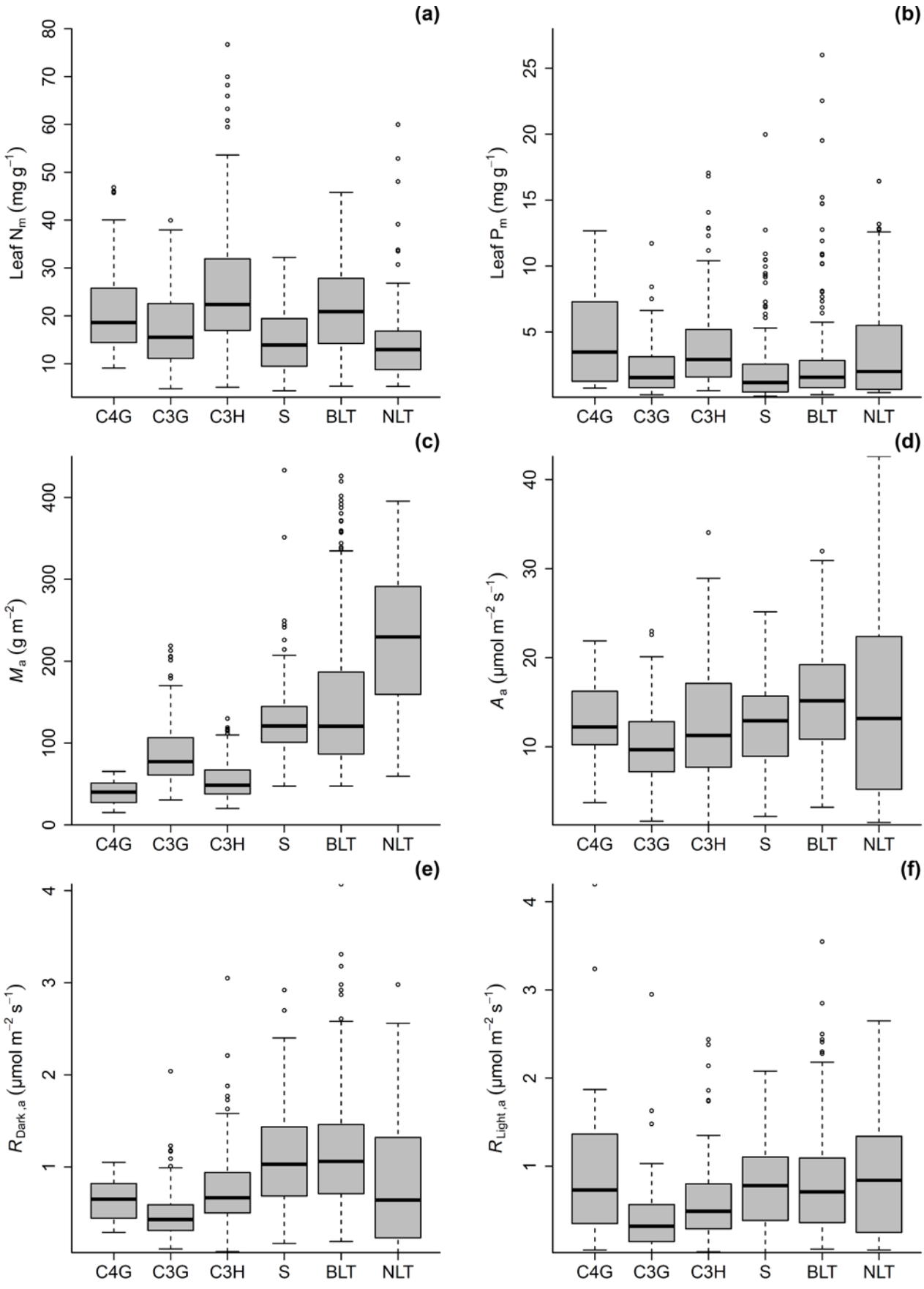

(a)
Leaf N_m (mg g^{-1})
10
20
30
40
50
60
70
80
C4G
C3G
C3H
S
BLT
NLT
(b)
Leaf P_m (mg g^{-1})
5
10
15
20
25
C4G
C3G
C3H
S
BLT
NLT
(c)
M_a (g m^{-2})
0
100
200
300
400
C4G
C3G
C3H
S
BLT
NLT
(d)
A_a (µmol m^{-2} s^{-1})
10
20
30
40
C4G
C3G
C3H
S
BLT
NLT
(e)
$R_{Dark,a}$ (µmol m^{-2} s^{-1})
1
2
3
4
C4G
C3G
C3H
S
BLT
NLT
(f)
$R_{Light,a}$ (µmol m^{-2} s^{-1})
1
2
3
4
C4G
C3G
C3H
S
BLT
NLT

Figure 3

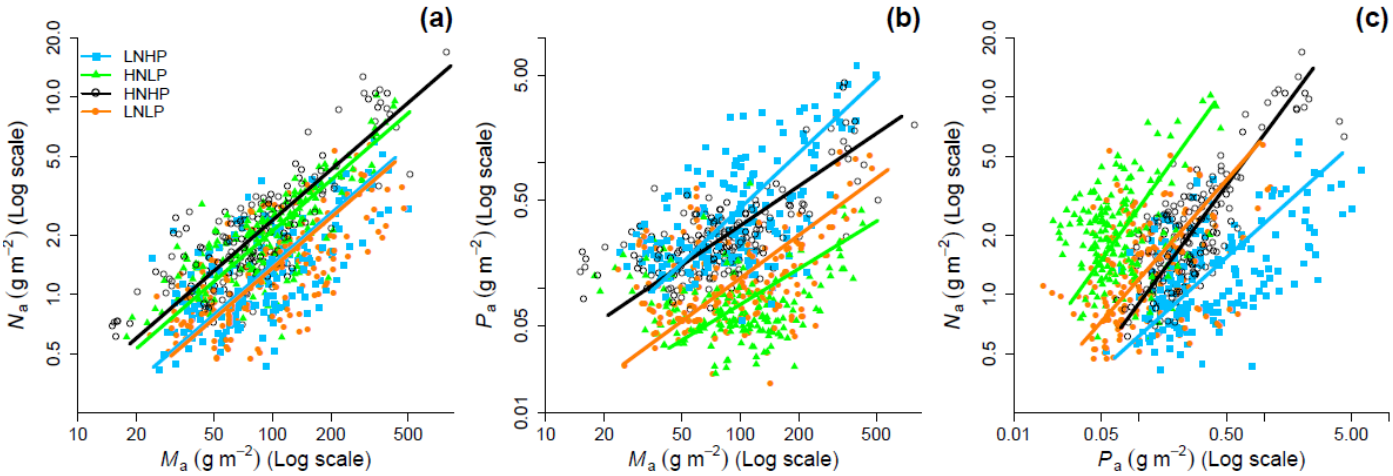

(a)
(b)
(c)
LNHP
HNLP
HNHP
LNLP
N_a (g m^{-2}) (Log scale)
P_a (g m^{-2}) (Log scale)
M_a (g m^{-2}) (Log scale)
0.5
1.0
2.0
5.0
10.0
20.0
0.01
0.05
0.50
5.00
10
20
50
100
200
500

Figure 4

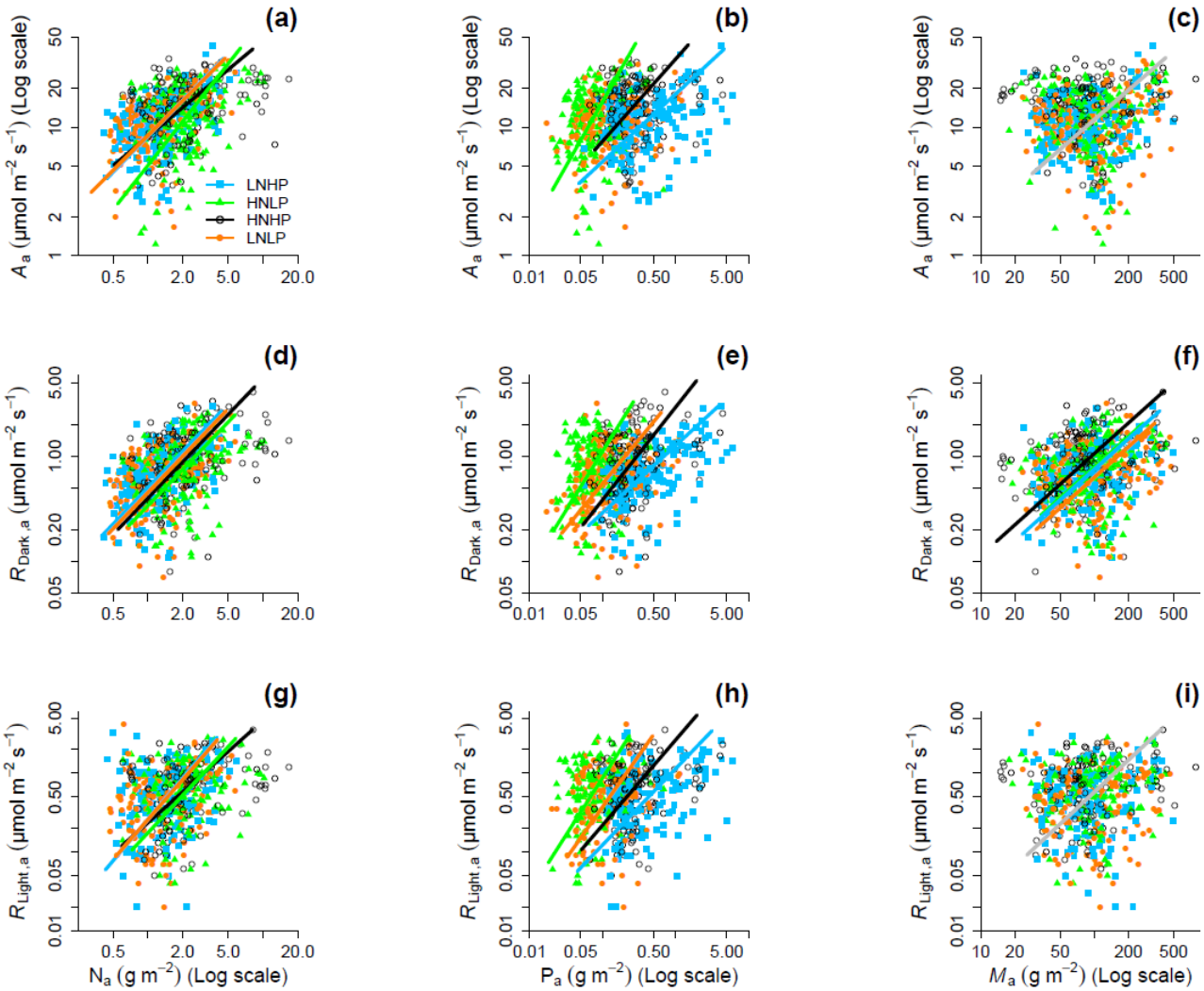
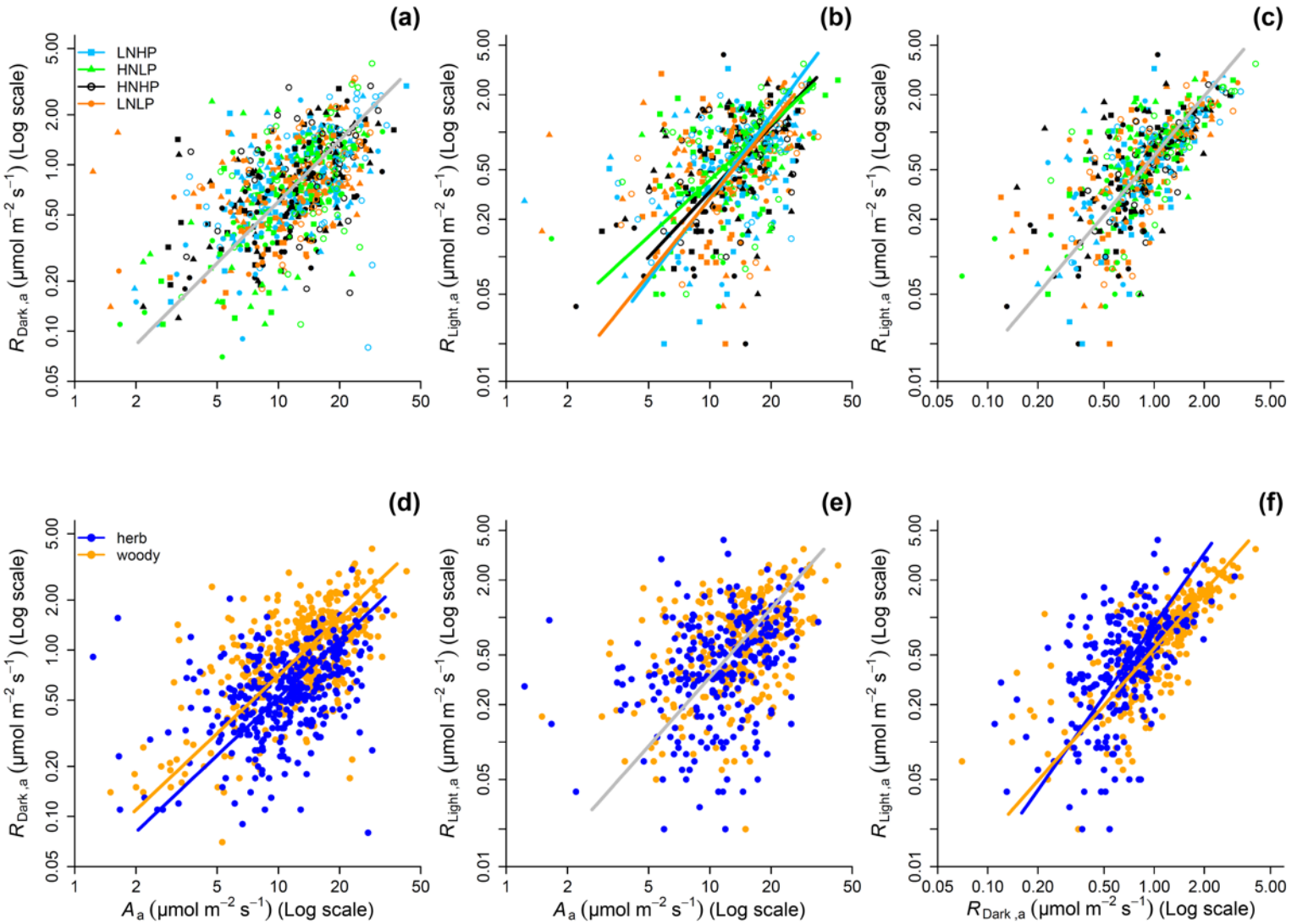

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
LNHP
HNLP
HNHP
LNLP
A_a (μmol m^{-2} s^{-1}) (Log scale)
$R_{Dark,a}$ (μmol m^{-2} s^{-1})
$R_{Light,a}$ (μmol m^{-2} s^{-1})
N_a (g m^{-2}) (Log scale)
P_a (g m^{-2}) (Log scale)
M_a (g m^{-2}) (Log scale)

Figure 5


(a)
(b)
(c)
(d)
(e)
(f)
LNHP
HNLP
HNHP
LNLP
herb
woody
$R_{Dark,a}$ (μmol m^{-2} s^{-1}) (Log scale)
$R_{Light,a}$ (μmol m^{-2} s^{-1}) (Log scale)
A_a (μmol m^{-2} s^{-1}) (Log scale)
$R_{Dark,a}$ (μmol m^{-2} s^{-1}) (Log scale)

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